Initial report of intratumoral tavokinogene telseplasmid with pembrolizumab in advanced melanoma: an approach designed to convert PD-1 antibody progressors into responders (NCT03132675)

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Victoria Atkinson[1], Andrew Haydon[2], Phillip Parente[3], Tom van Hagen[4], Gregory A. Daniels[5], Pablo Fernandez-Penas[6], Mecker Moller[7], Igor Puzanov[8], Sajeve Thomas[9], Robert H. Andtbacka[10], Clemens Krepler[11], Rachel Roberts-Thomson[12], Alain Algazi[13], Lauren Svenson[14], Erica Browning[14], David A. Canton[14], Christopher Twitty[14], Sharron E. Gargosky[14], Adil I. Daud[13]

[1]Princess Alexandra Hospital, Woolloongabba, AU; [2]The Alfred Hospital, Melbourne, AU; [3]Eastern Health Monash University, Melbourne, AU; [4]St John of God Hospital, Subiaco, AU; [5]University of California San Diego, San Diego, CA, USA; [6]Westmead Hospital, Sydney, AU; [7]University of Miami, Miami, USA; [8]Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA; [9]UF Health Cancer Center at Orlando Health, Orlando, USA; [10]University of Utah, Salt Lake City, USA; [11]Merck & Co., Inc., Kenilworth, NJ, USA; [12]Calvary North Adelaide Hospital, North Adelaide, AU; [13]UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, USA; [14]OncoSec Medical Incorporated, San Diego, CA, USA

INTRODUCTION

- The combination of local intratumoral administration of tavokinogene telseplasmid (interleukin-12 [IL-12] plasmid) followed by electroporation (collectively, TAVO™) with the anti-programmed cell death protein 1 (anti–PD-1) antibody pembrolizumab (KEYTRUDA®) has been granted fast-track designation for evaluation for accelerated approval for patients with FDA-approved anti–PD-1 antibody-refractory melanoma.
- Anti–PD-1 antibodies, such as pembrolizumab or nivolumab, are the mainstay of treatment for advanced melanoma.[1,2]
  - However, their clinical benefit is limited by PD-1 resistance in ~65% of treated patients,[3,4] and absence of T-cell–inflamed tumors has emerged as a key determinant of poor response[5]
- TAVO™ administration into accessible lesions induces expression of the proinflammatory cytokine IL-12, with minimal systemic immune-related toxicity.[6,7]
  - Via this mechanism, TAVO™ is able to convert treated and non-treated non-immunogenic tumors into highly inflamed, immunologically active lesions
  - The combination of TAVO™ and pembrolizumab has led to durable remissions in patients with low tumor-infiltrating lymphocytes (TIL) tumors
- The purpose of this study (KEYNOTE-695) is to determine the ability of TAVO™ to reverse anti–PD-1 resistance in stage III/IV metastatic melanoma whose tumors have unequivocally progressed after a full course of FDA-approved anti–PD-1 therapies.

OBJECTIVES

PRIMARY OBJECTIVE

- To assess the efficacy of TAVO™ combined with pembrolizumab in patients with advanced melanoma. Eligible patients had refractory, locally advanced or metastatic disease defined as unresectable stage III/IV metastatic melanoma that had definitively progressed on a full-course of anti–PD-1 treatment with pembrolizumab or nivolumab (OPDIVO®).
- The primary endpoint is the best overall response rate (BORR) according to Response Evaluation Criteria In Solid Tumors (RECIST) v1.1, measured by radiologic assessment, with confirmation by independent review.

SECONDARY OBJECTIVES

- To evaluate safety and tolerability of the combined therapy.
- To assess duration of response, objective response rate, BORR, progression-free survival (PFS), immune PFS, and overall survival of the combined therapy.

EXPLORATORY OBJECTIVE

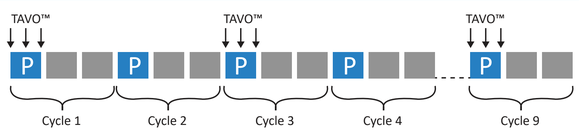
- To evaluate peripheral and intratumoral treatment-related immunologic changes in responding and non-responding patients.

MATERIALS AND METHODS

STUDY DESIGN AND INTERVENTIONS

- KEYNOTE-695 is an ongoing, global, registration-directed multicenter Phase 2b, open-label study (NCT03132675) of TAVO™ with intravenous (IV) pembrolizumab in patients with unresectable, advanced melanoma.
- Eligible patients have refractory, locally advanced or metastatic disease defined as unresectable stage III or stage IV metastatic melanoma, which had definitively progressed on full course of anti–PD-1 treatment with pembrolizumab or nivolumab. Key eligibility criteria, including the definitive nature of anti–PD-1 failure, are included in **Figure 1**.
- Patients are treated with 0.5 mg/mL TAVO™ to the accessible lesions on days 1, 5, and 8 every 6 weeks, and with 200 mg IV pembrolizumab on day 1 of each 3-week cycle for 27 weeks (**Figure 1**).
  - TAVO™ is injected at a dose volume of ~¼ of the calculated lesion volume, with a minimum dose volume/lesion of 0.1 mL. An applicator with a hexagonal array of 6 microneedles is placed into and around the injected tumor, with the tip co-localized at the site(s) and depth of plasmid injection. Six pulses at a field strength of 1500 volt/cm and pulse width of 100 µs at 300-msec intervals are delivered
  - Pembrolizumab is administered using a 30-minute (-5/+10 min) infusion
  - When feasible, at least 1 distal, non-target lesion remains untreated with TAVO™

Figure 1. Study design



Key eligibility criteria

- Patients (≥18 years) with stage III/IV unresectable melanoma who progressed on pembrolizumab or nivolumab treatment
- ECOG performance status of 0–1
- At least 1 anatomically distinct lesion, EP-accessible and accurately measured in at least 1 dimension
- Patients must have failed all available treatment options (BRAF inhibitors and other targeted therapies)
- Patients must be definitive anti–PD-1 non-responders:
  - Definitively progressed on a full course (4+ doses) of anti–PD-1 treatment with pembrolizumab or nivolumab
  - Progressive disease per RECIST v1.1 determined by radiologic assessment
  - Documented disease progression ≤24 weeks of the last dose of anti–PD-1
  - No intervening therapies between anti–PD-1 failure and TAVO™ + KEYTRUDA® (pembrolizumab)

BRAF, B-Raf murine sarcoma viral oncogene homologue; ECOG, Eastern Cooperative Oncology Group; EP, electroporation; PD-1, programmed cell death protein 1; P, pembrolizumab; RECIST, Response Evaluation Criteria In Solid Tumors; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation.

- Patients are eligible to receive the combined treatment for up to 35 cycles of KEYTRUDA® (pembrolizumab) from baseline or until subsequent disease progression.

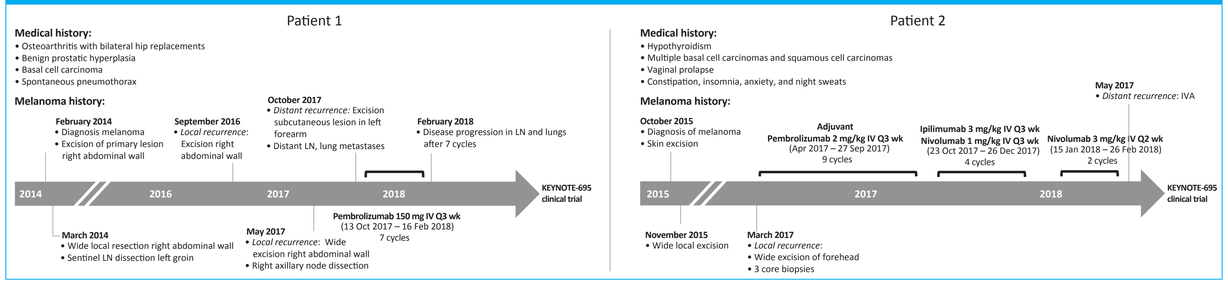
ASSESSMENTS

- Efficacy outcomes: BORR was assessed at 12 weeks using the RECIST v1.1.
- For multispectral immunohistochemistry and flow cytometry assessments, fixed and fresh tumor biopsies, respectively, were collected at screening and on day 1 of cycle 2.
- Immune subsets pre-/post-treatment were assessed by multicolor flow cytometry; serum analysis was performed using the MILLIPLEX MAP human high sensitivity T cell panel.
- Intratumoral gene expression of individual genes was analyzed with PanCancer IO 360™ Gene Expression Panel, and quantitation was performed using the nSolver Software.
- Safety was assessed by frequency and severity of adverse events (AEs) and serious AEs (SAEs), as well as relationship to treatment.

PATIENT HISTORY

- Representative medical and melanoma histories of 2 patients enrolled in the KEYNOTE-695 study, and who developed a response to the TAVO™/KEYTRUDA® combination are depicted in **Figure 2**.

Figure 2. Medical and melanoma history for the 2 patients who subsequently developed a response to the TAVO™/KEYTRUDA® combination



IV, intravenous; LN, lymph node; Q2 wk, every 2 weeks; Q3 wk, every 3 weeks.

RESULTS

PATIENTS

- As of Sept. 1, 2018, 21 patients had been enrolled in the study. Out of the 21 patients, 9 patients had completed 12 weeks of treatment and reached the first tumor evaluation point at approximately 12 weeks, while the remaining 12 patients did not yet reach the first tumor evaluation (**Table 1**).
- All 9 patients were previously treated and definitively progressed on anti–PD-1 therapies, with 56% (5/9) having had more than 1 prior line of therapy.

Table 1. Patient demographics and baseline characteristics

Characteristic	Overall population (N = 21)	Population who reached initial tumor assessment (~12–15 weeks) post-completion of 2 cycles of TAVO™ (N = 9)
Mean age, years (SD)	64 (12.0)	64 (11.1)
Male, n (%)	15 (71.4)	6 (66.6)
Race, n (%)		
Caucasian	21 (100.0)	9 (100.0)
Prior lines of systemic therapy, n (%)		
1	13 (61.9)	4 (44.4)
2	4 (19.0)	2 (22.2)
>2	4 (19.0)	3 (33.3)
Number of prior anti–PD-1 cycles	4–18	4–18
Pembrolizumab IV	4–18	4–18
Nivolumab IV	4–16[a]	4–16[a]
Stage at enrollment, n (%)		
III	3 (14.3)	0 (0.0)
IVA	10 (47.6)	4 (44.4)
IVB	5 (23.8)	4 (44.4)
IVC	3 (14.3)	1 (11.1)

[a]One patient (patient 5) received 3 nivolumab IV treatments: for 8 cycles, for 4 cycles, and once.
IV, intravenous; PD-1, programmed cell death protein 1; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation; SD, standard deviation.

RESPONSE

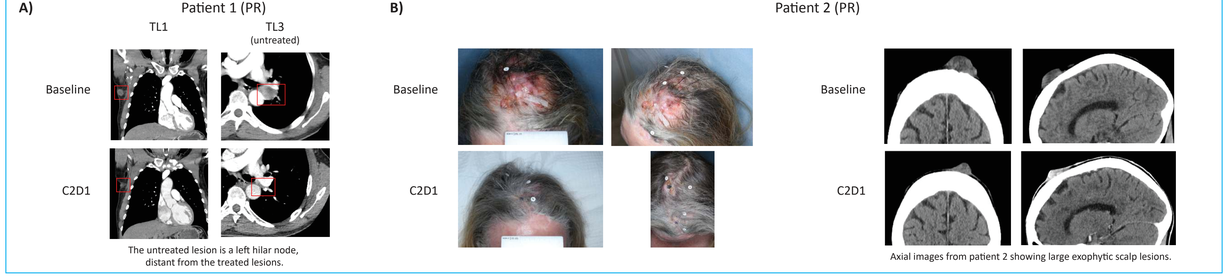
- Tumor responses for the first 9 patients at initial tumor assessment (~12–15 weeks) are presented in **Table 2**.
- Two of the 9 assessed patients had PR; reductions in tumor size were observed in both treated and untreated lesions of both patients (**Figure 3**). One of the 9 patients had SD.

Table 2. Summary of response for the patients who completed 12 weeks of treatment

Patient number	Prior anti–PD-1 treatments (no. of cycles)	Definitive progression on prior anti–PD-1 treatment	Population who reached initial tumor assessment (~12–15 weeks) post-completion of 2 cycles of TAVO™ (N = 9)
1	Pembrolizumab IV (7)	YES	PR
2	Adjuvant pembrolizumab IV (9) Nivolumab IV (4) Nivolumab IV (4)	YES	PR
3	Pembrolizumab IV (10)	YES	SD
4	Pembrolizumab IV (4)	YES	iUPD; iSD (SD TL / new NTL)
5	Nivolumab IV (8) Pembrolizumab IV (4) Nivolumab IV (4) Nivolumab IV (once)	YES	iUPD; WDC (PR TL / new NTL)
6	Pembrolizumab IV (18)	YES	iUPD; WDC (SD TL / new NTL)
7	Pembrolizumab IV (4)	YES	PD
8	Pembrolizumab IV (11) Nivolumab IV (16)	YES	PD
9	Pembrolizumab IV (7)	YES	PD

IV, intravenous; iSD, immunologic stable disease; iUPD, immunologic unconfirmed progressive disease; NTL, non-target lesion; PD, progressive disease; PD-1, programmed cell death protein 1; PR, partial response; SD, stable disease; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation; TL, target lesion; WDC, withdrew consent.

Figure 3. Representative target and non-target A) lesion images, and B) computed tomography axial images of the 2 responder patients, at screening and on day 1 of cycle 2 with TAVO™

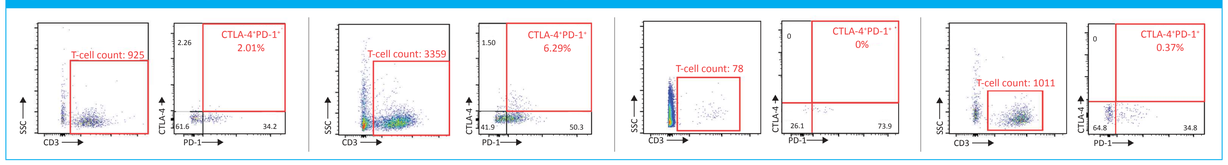


C2D1, cycle 2 day 1; PR, partial response; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation; TL, target lesion.

IMMUNE MONITORING

- At screening, patients had a very low frequency of CD8⁺ partially exhausted cytotoxic T lymphocytes (peCTLs [PD-1⁺CTLA-4⁺]; **Figure 4**).

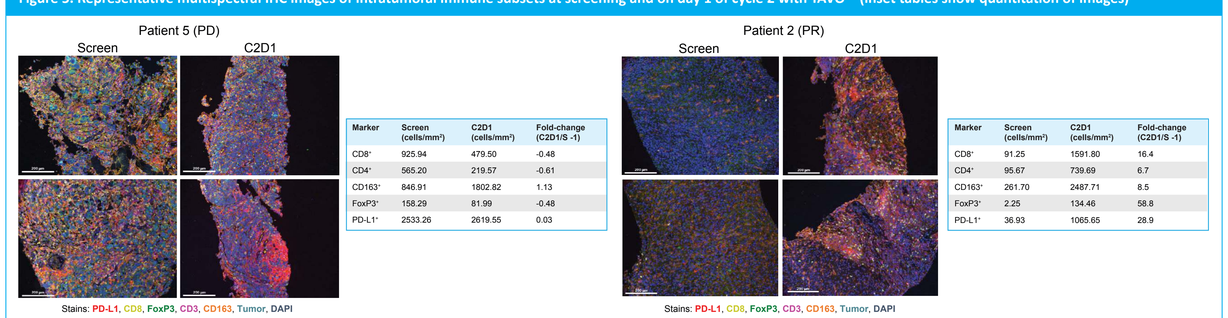
Figure 4. Flow cytometry images showing immunologically devoid tumors at screening in the first 4 patients who provided fresh (non-FFPE) tumor biopsies



Gating strategy: peCTLs: singles> live> CD45⁺ >CD3⁺CD8⁺ >CTLA-4⁺PD-1⁺
CD, cluster of differentiation; CTLA-4, cytotoxic T-lymphocyte–associated protein 4; FFPE, formaldehyde fixed-paraffin embedded; PD-1, programmed cell death protein 1; SSC, side scatter.

- Following 1 cycle of combined therapy, a significant treatment-related increase in TIL density was observed in responder vs non-responder patients (**Figures 5** and **6**).

Figure 5. Representative multispectral IHC images of intratumoral immune subsets at screening and on day 1 of cycle 2 with TAVO™ (inset tables show quantitation of images)

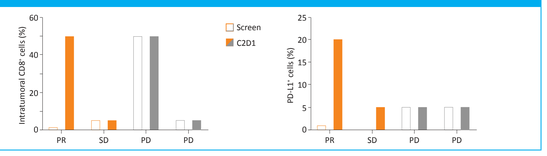


Marker	Screen (cells/mm²)	C2D1 (cells/mm²)	Fold-change (C2D1/S -1)
CD8⁺	925.94	479.50	-0.48
CD4⁺	565.20	219.57	-0.61
CD163⁺	846.91	1802.82	1.13
FoxP3⁺	158.29	81.99	-0.48
PD-L1⁺	2533.26	2619.55	0.03

Marker	Screen (cells/mm²)	C2D1 (cells/mm²)	Fold-change (C2D1/S -1)
CD8⁺	91.25	1591.80	16.4
CD4⁺	95.67	739.69	6.7
CD163⁺	261.70	2487.71	8.5
FoxP3⁺	2.25	134.46	58.8
PD-L1⁺	36.93	1065.65	28.9

C2D1, cycle 2 day 1; CD, cluster of differentiation; DAPI, 4',6-diamino-2-phenylindole; FoxP3, foxhead box P3; IHC, immunohistochemistry; PD, progressive disease; PD-L1, programmed cell death ligand 1; PR, partial response; S, screening; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation.

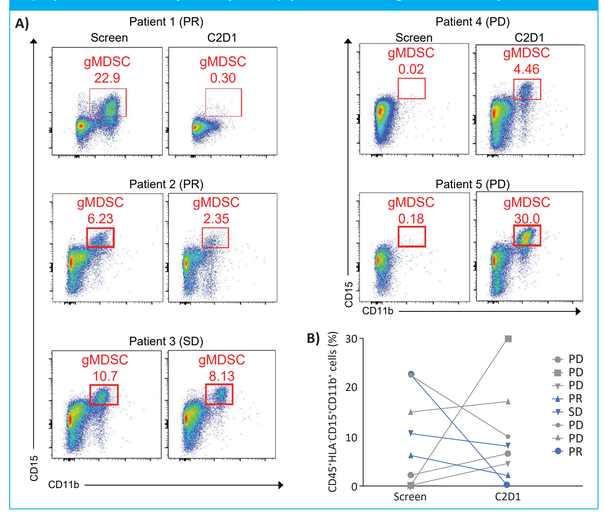
Figure 6. Enumeration of intratumoral CD8⁺ and PD-L1⁺ cells by immunohistochemistry



C2D1, cycle 2 day 1; CD, cluster of differentiation; PD, progressive disease; PD-L1, programmed cell death ligand 1; PR, partial response; SD, stable disease.

- In responder patients, peripheral immune-suppressive granulocytic myeloid-derived suppressor cells (gMDSCs) were decreased after 1 cycle of combined therapy (**Figure 7**).

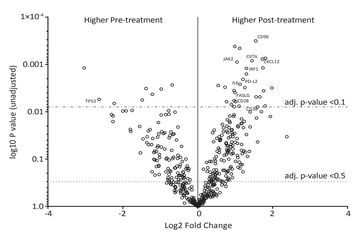
Figure 7. Peripheral gMDSCs at screening and on day 1 of cycle 2 with TAVO™: A) representative flow cytometry, and B) quantification of gMDSCs in all 9 patients



Gating strategy gMDSCs: singles >live >CD45⁺ >CD3⁻CD19⁻/HLA⁻ >CD15⁺CD11b⁺
C2D1, cycle 2 day 1; CD, cluster of differentiation; gMDSC, granulocytic myeloid-derived suppressor cell; HLA, human leukocyte antigen; PD, progressive disease; PR, partial response; SD, stable disease; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation.

- A treatment-related upregulation of immune-based transcripts in the tumor micro-environment was observed in 7 matched biopsies (**Figure 8**).

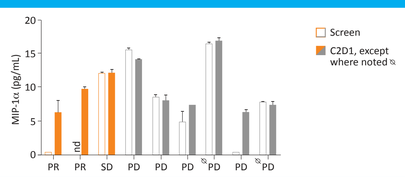
Figure 8. Volcano plot representing transcriptional analysis of biopsies collected at screening and on day 1 of cycle 2 with TAVO™



C2D1, cycle 2 day 1; CD, cluster of differentiation; CIITA, class II, major histocompatibility complex transactivator; CXCL12, C-X-C motif chemokine ligand 12; FASLG, FAS ligand gene; IL6, interleukin 6; IRF1, interferon-regulatory factor 1; JAK2, Janus kinase 2; PD-L2, programmed cell death ligand 2; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation; TP53, tumor protein p53.

- Patients who partially responded to therapy showed a treatment-related increase in serum levels of macrophage inflammatory protein 1-alpha (MIP-1α; **Figure 9**).

Figure 9. Serum levels of MIP-1α at screening and on day 1 of cycle 2 with TAVO™



C2D1, cycle 2 day 1; MIP-1α, macrophage inflammatory protein 1-alpha; nd, not determined; PD, progressive disease; PR, partial response; SD, stable disease; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation.

SAFETY

- TAVO™-related AEs were limited to grade 1 injection site discomfort/pain, except for one grade 3 episode of cellulitis, which resolved completely (**Table 3**).

Table 3. Numbers of patients with adverse events in the patient population who completed 12 weeks of treatment

	TAVO™-related AEs	Pembrolizumab-related AEs	Total AEs/SAEs related to study drugs
Grade 1, n (%)	5/9 (55.6)	3/9 (33.3)	8/9 (88.9)
Grade 3, n (%)	1/9 (11.1)*	2/9 (22.2)	3/9 (33.3)

*Cellulitis, cleared.
AE, adverse event; TAVO™, intratumoral administration of interleukin-12 plasmid followed by electroporation; SAE, serious adverse event.

CONCLUSIONS

- TAVO™ and pembrolizumab are well tolerated, with nearly all grade 1 study-related AEs.
- This registration-directed study demonstrated that patients who definitively progressed on multiple courses of anti–PD-1 treatment with pembrolizumab or nivolumab experienced substantial reductions in both treated and untreated tumors, with a 22.2% BORR (2/9) and a 33.3% disease control rate (DCR; 3/9).
- The intratumoral treatment-related increase in TIL density, accompanied upregulated immune-based transcripts in the tumor, as well as reduced frequencies of gMDSCs in the periphery and increased MIP-1α in the serum, collectively, are evidence of strong systemic immune response following TAVO™ / pembrolizumab combination in patients who definitively failed prior treatment with either pembrolizumab or nivolumab.
- Early clinical and immunologic evidence of reversing anti–PD-1 resistance demonstrates the potential of TAVO™ to convert definitive pembrolizumab or nivolumab progressors into responders.

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