Intratumoral delivery of tavokinogene telseplasmid yields systemic immune responses in metastatic melanoma patients

A. Algazi1, S. Bhatia2, S. Agarwala3, M. Molina4, K. Lewis5, M. Faries6, L. Fong7, L. P. Levine1, M. Franco1, A. Oglesby1, C. Ballesteros-Merino1, C. B. Bifulco7, B. A. Fox8, D. Bannavong8, R. Talia8, E. Browning8, M. H. Le8, R. H. Pierce8, S. Gargosky8, K. K. Tsai1, C. Twitty8 & A. I. Daud1

1Department of Medicine, University of California, San Francisco, San Francisco; 2Department of Medicine, University of Washington, Seattle; 3St. Luke’s Cancer Center, Bethlehem; 4Lakeland Health Medical Center, Lakeland; 5University of Colorado Cancer Center — Anschutz, Denver; 6Providence John Wayne Cancer Institute, Santa Monica; 7Earle A. Chiles Research Institute at Providence Portland Medical Center, Portland; 8OncoSec Medical Incorporated, San Diego, USA

Available online XXX

INTRODUCTION

The cytokine interleukin 12 (IL-12) occupies a unique niche in the cytokine repertoire bridging the innate and adaptive immune systems. IL-12 is typically triggered upon pathogen-associated molecular pattern or danger-associated molecular pattern recognition and causes secretion of interferon-γ (IFN-γ) by T cells, natural killer (NK) cells, and dendritic cells (DCs), which in turn causes additional IL-12 production by immune cells. IL-12 causes Th1 polarization, reduces regulatory T cells, and converts myeloid-derived suppressor cells to functional DCs. In addition, IL-12 (and IFN-γ) are crucial third signals sent by cross-presenting DC (cDC1) to naive CD8+ T cells, aiding their transformation into effector T cells. Intravenous recombinant IL-12 (rIL-12) has shown clinical efficacy in solid tumor malignancies including renal cell cancer and melanoma, albeit with a high level of serious adverse events (AEs). Subcutaneous and intraläsional recombinant cytokines have a lower toxicity, but also a much lower efficacy. In contrast, intraläsional and systemic rIL-12, intratumoral injection of plasmid encoding IL-12 (Tavo) leads to sustained cytokine elaboration in the tumor microenvironment in vivo, with minimal systemic exposure. In the syngeneic B16 melanoma model, local IL-12 plasmid electroporation causes regression of both established local
and distant (non-treated) lesions, while yielding immune memory to tumor rechallenge. A phase I clinical trial of IL-12 plasmid electroporation established a biologically effective dose and demonstrated the safety of this approach, as well as its preliminary efficacy in increasing intratumoral IL-12 and IFN-γ, yielding sustained, global remissions in several patients after one cycle of therapy. We evaluated Tavo for efficacy and safety in an open-label, phase II trial.

METHODS

Study design

This was a prospective, multicenter, open-label, phase II trial (NCT01502293) evaluating the clinical efficacy and safety of Tavo in melanoma patients.

Patients

Eligible patients were required to be ≥18 years old with pathologically documented melanoma, with Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and an unresectable American Joint Committee on Cancer (AJCC) stage IIIB, IIIC, or IV A, B, or C, and two or more melanoma lesions accessible to electroporation. Any prior therapy was permitted. Any treatment-related toxicities resolved to grade 1 or better before study treatment.

Treatment

Tavo (IL-12 plasmid, 0.5 mg/ml) was administered on days 1, 5, and 8 of each 90-day cycle (Figure 1) by intratumoral injection at a dose volume of one-quarter of the calculated lesion volume (minimum = 0.1 ml). Electroporation was carried out using six pulses of 1500 V/cm and a pulse width of 100 μs at 1-second intervals (ImmunoPulse, OncoSec Medical, Inc. San Diego, CA). Additional patients treated with the same plasmid dose but on different schedules (dose schedule exploration, supplementary Figure S1, available at Annals of Oncology online) were included in the translational and untreated lesion response analyses.

Efficacy assessment

Tumor lesions and tumor response were assessed by the investigator according to a modified version of RECIST version 1.0 that allowed inclusion of any number of skin lesions >0.3 cm at the largest diameter to be followed as target lesions, inclusion of latent responses, and assessment of the net tumor burden in the setting of new lesions. Progression-free survival was assessed as the time from the first day of study treatment until the time that the sum of the diameters of all measurable lesions increased by at least 30% from baseline. Additional information regarding response measures for treated and untreated lesions is provided in the supplementary Materials, available at Annals of Oncology online.

Safety evaluation

Safety was assessed by monitoring AEs, pain assessments, clinical laboratory tests, and vital signs. AEs were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0.

Translational medicine and statistical plan

See the supplementary Materials, available at Annals of Oncology online.

RESULTS

Baseline patient characteristics

For the main study, 38 patients were consented and 30 were eligible for the study and received at least one dose of treatment (Table 1). Of these, 28 patients were assessable for response (one withdrew before post-treatment assessment, one was deemed ineligible after initiation of treatment). Prior exposure to immunotherapy included 13 patients treated previously with systemic cytokines (high dose IL-2 or IFNα-2a), nine patients treated with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibodies and four patients (13.3%) treated with anti-programmed cell death protein 1 (PD-1) antibodies. In

![Figure 1](https://doi.org/10.1016/j.annonc.2019.12.008) Volume xxx ■ Issue xxx ■ 2020

**Figure 1.** (A) CONSORT diagram with the screening and treatment assignments of patients consented to study. (B) Tavokinogene telseplasmid (0.5 mg/ml) was injected at a dose-volume of one-quarter of the calculated lesion volume. Patients were treated on days 1, 5, and 8 of every 90-day treatment cycle. Tumor response assessments were made every 90 days.

EP, electroporation; i.t., intratumoral.
addition, 24 patients were screened and 21 additional patients were treated in the schedule exploration cohorts (demographics are described in supplementary Table S1, available at Annals of Oncology online).

### Table 1. Patient demographics and patient history

<table>
<thead>
<tr>
<th>Category</th>
<th>Toxicity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>≥ Grade 3</th>
<th>All grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Nausea</td>
<td>10 (33.3%)</td>
<td>15 (50.0%)</td>
<td>4 (13.3%)</td>
<td>29 (96.7%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Vomiting</td>
<td>2 (6.7%)</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>1 (3.3%)</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td>Constitutional</td>
<td>Fatigue</td>
<td>4 (13.3%)</td>
<td>1 (3.3%)</td>
<td>–</td>
<td>5 (16.7%)</td>
</tr>
<tr>
<td></td>
<td>Injection site discoloration</td>
<td>4 (13.3%)</td>
<td>–</td>
<td>–</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td></td>
<td>Injection site inflammation</td>
<td>3 (10.0%)</td>
<td>1 (3.3%)</td>
<td>–</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td></td>
<td>Chills</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td></td>
<td>Injection site discharge</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td></td>
<td>Injection site erythema</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td></td>
<td>Edema peripheral</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td></td>
<td>Pain</td>
<td>–</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Infectious</td>
<td>Pyrexia</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Procedural</td>
<td>Cellulitis</td>
<td>–</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Procedural pain</td>
<td>23 (76.7%)</td>
<td>–</td>
<td>1 (3.3%)</td>
<td>24 (80.0%)</td>
</tr>
<tr>
<td></td>
<td>Pain in extremity</td>
<td>4 (13.3%)</td>
<td>1 (3.3%)</td>
<td>–</td>
<td>5 (16.7%)</td>
</tr>
<tr>
<td></td>
<td>Arthralgia</td>
<td>2 (6.7%)</td>
<td>1 (3.3%)</td>
<td>–</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td></td>
<td>Muscle spasms</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td></td>
<td>Musculoskeletal stiffness</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Neoplasms</td>
<td>Neoplasms NOS</td>
<td>–</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Nervous system</td>
<td>Headache</td>
<td>4 (13.3%)</td>
<td>1 (3.3%)</td>
<td>–</td>
<td>5 (16.7%)</td>
</tr>
<tr>
<td></td>
<td>Dizziness</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular accident</td>
<td>–</td>
<td>–</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>Anxiety</td>
<td>1 (3.3%)</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Cough</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>Pruritus</td>
<td>2 (6.7%)</td>
<td>1 (3.3%)</td>
<td>–</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td></td>
<td>Rash</td>
<td>3 (10.0%)</td>
<td>–</td>
<td>–</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td></td>
<td>Ecchymosis</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td></td>
<td>Skin disorder</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Vascular</td>
<td>Lymphedema</td>
<td>2 (6.7%)</td>
<td>–</td>
<td>–</td>
<td>2 (6.7%)</td>
</tr>
</tbody>
</table>

**AEs**

All treatment-emergent AEs (TEAEs) regardless of attribution observed in at least two patients and grade 3 or higher AEs are described in Table 2. Transient procedural pain (n = 24, 80%) and injection site reactions were common. Constitutional symptoms were observed in a minority of patients including fatigue (n = 5, 16.7%), pyrexia (n = 2, 6.7%), and chills (n = 2, 6.7%). Grade 3 TEAEs were limited to transient procedural pain (n = 1, 3.3%) and a cerebrovascular accident that was determined to be unrelated to treatment on study. A patient in one of the schedule exploration cohorts also had grade 3, treatment-related cellulitis (supplementary Table S2, available at Annals of Oncology online).

### Clinical response

The best overall response rate at any time point for Tavo treatment in the main study population (cohort A) was 35.7% (waterfall plot, Figure 2A). Seven patients had disease progression before the first response assessment at 90 days and are represented as having a 100% change in tumor burden for graphic purposes. Responses included five complete responses and responses in patients with extensive in-transit/satellite metastases (Figure 2E and F). The clinical response rate was 26.7% in cohort B (4/15) and none of the four patients treated in cohort C responded. The best overall response rate for patients in all cohorts was
29.8% (supplementary Figure S2, available at Annals of Oncology online). Per-patient lesion and response data are presented in supplementary Table S3, available at Annals of Oncology online.

Adaptive resistance and response to checkpoint therapy

The median progression-free survival was 3.72 months (95% confidence interval (CI) 0.55–6.89), 3.2 months (95% CI 2.41–3.97), and 2.5 months (95% CI not defined) in cohorts A (main study), B, and C, respectively. The median overall survival was not reached in any cohort (Figure 2C and D, supplementary Figure S2B, available at Annals of Oncology online). Treatment with Tavo induced adaptive resistance as demonstrated by increases in programmed death-ligand 1 (PD-L1) expression by immunohistochemistry (Figure 3A). As a possible consequence, although durable responses were seen in four patients, transient responses were observed in six others (Figure 2B). In some patients, however, Tavo increased the total number of tumor infiltrating lymphocytes and the CD8:FoxP3 ratio suggesting an increase in the relative abundance of effector T cells versus regulatory cells (e.g. Figure 3C–E), and, in a retrospective analysis, six of eight patients progressing on Tavo responded to pembrolizumab immediately thereafter (Figure 3B, F–J).
Inflammatory gene expression

Tavo induced significant increases in multiple immune transcripts (Figure 4A), including modules associated with immune activation (Figure 4B), NK cell activity, antigen presentation and adaptive resistance (Figure 4C), as well as T cell trafficking (Figure 4D); all characteristic of an...
Intratumoral immune response

Higher pre-treatment

Log10 P-value

1 × 10^{-7}

1 × 10^{-6}

1 × 10^{-5}

1 × 10^{-4}

1 × 10^{-3}

1 × 10^{-2}

1 × 10^{-1}

Log2 fold change

HLA-DQB1
HLA-DQA1
HLA-C
HLA-B
ZAP70
CCL5
GZMAT
GFB1
JAK3
S100A8
S100A9
PRF1
TNFSF10
JAK1

Adj. P value <0.01

Adj. P value <0.05

Adj. P value <0.1

Immune activation

B

CD8a
CD9b
STAT4
IL2RB
IL12RB1
GZMA

Natural killer

C

KLRB1
KLRK1
CIITA
PSMB7
B7TF3
TAPBP
CD96
PDL1
TRAL
TGFB1

Antigen presentation

Adaptive resistance

Trafficking

D

CXCL2
CXCR3
VCAM1
CCR5
CCL5
SELL

All patients

Clinical benefit

Progressive disease

E

IFN-γ pathway score

Pre-treatment

Post-treatment

Pre-treatment

Post-treatment

Pre-treatment

Post-treatment

Pre-treatment

Post-treatment

Pre-treatment

Post-treatment

IFN-γ pathway score

ns
antitumor immune response. Specific findings included increased expression of CD3E, CD8, STAT4, IL-2RB and IL-12RB1 as well as effector molecules such as GZMA, consistent with the known effect of IL-12 on T cell and NK cell activation. A significant increase in transcripts associated with cross-presenting DCs such as CIITA, BATF3, PSMB7 and TAPBP were also noted. Additionally, the chemokine receptor CXCR3, expressed on TH1-polarized T cells, as well as chemokines and adhesion molecules were significantly increased. However, this global increase in genes associated with productive antitumor immunity was accompanied by increases in genes associated with adaptive resistance including CD274 (PD-L1), TRFB1 and TRAIL. IFN-γ gene expression increased overall in patients benefitting from treatment, but not in patients with progressive disease as the best treatment response (Figure 4E). Overall, these results suggest that Tavo induced NK cell and DC activation, recruitment, and activation of CD4+ T cell and CD8+ T cells, as well as the compensatory development of adaptive resistance.

Systemic immune response
We assessed systemic immune activity after treatment with Tavo. Analysis of serum inflammatory markers showed an increase in IL-1β and MIP-1α as well as the proportion of proliferating effector T cells in the periphery in responding patients. Regression of at least one untreated lesion was observed in 46% of patients.

Figure 4. Tavokinogene telseplasmid-induced productive antitumor immune responses. (A) Volcano plot of both non-responding and responding patients based on transcriptional analysis of biopsies collected at screening and post-treatment. In particular, intratumoral expression of genes associated with (B) immune activation (C) natural killer (NK) cell activity, antigen presentation, adaptive resistance, and (D) T cell trafficking was increased after treatment. (E) Interferon-γ gene expression increased overall and in patients benefitting from treatment, but not in patients with progressive disease as the best treatment response (n = 28 including 14 patients with pre-/post-biopsy specimens).

Figure 5. Signs of systemic immune activity after treatment with tavokinogene telseplasmid (Tavo). Tavo increased circulating levels of (A) IL-1β and (B) MIP-1α as well as (C) the proportion of proliferating effector T cells in the periphery in responding patients. (D) Tavo increased the frequency of NK cells in the periphery in the responding population. (E) Best overall response in treated and (F) untreated lesions as assessed as the sum of diameters of all lesions in each category for patients treated in the main study and in the additional cohorts (B and C). Regression of at least one untreated lesions was observed in 46% of patients.
NK cells (CD56dimCD16+, Figure 5D) in responding but not in non-responding patients (P < 0.05).

For patients in the main study and expansion cohorts, responses in untreated lesions were common. In 40 patients with uninjected pre- and post-treatment tumor measurements available, the best overall response for untreated lesions was 25% (n = 40, Figure 5F) compared with a 43.8% response rate in treated lesions (n = 45 patients, Figure 5E). The per-lesion response rate for treated lesions was 62.7% (64/102) and for untreated lesions it was 17.4% (20/115).

DISCUSSION

Prior therapeutic approaches to rIL-12, including intraluminal and systemic administration, have had limited efficacy due to transient exposures associated with intraluminal therapy and severe toxicity associated with systemic administration. We previously described a phase I intratumoral dose-escalation rIL-12 electroporation trial demonstrating that a plasmid concentration of 0.5 mg/ml was well tolerated and showed clinical effectiveness with absent or minimal toxicity and systemic immune activation. In the current report, we confirm these findings in a phase II expansion, demonstrating a 35.7% overall response rate in the main study and a 29.8% overall response rate in all cohorts.

Recently, several intratumoral therapies have been explored, either in combination or alone, with a goal of demonstrating that an ‘in situ’ immunization strategy can yield systemic immune effects. For example, a retrospective analysis of the modified herpes virus, Talimogene lahrenparevec, administered intratumorally, demonstrated an objective response rate of 26%, with regression of some baseline uninjected lesions. In the current phase II trial of Tavo, we used different response criteria, but regression of treated lesions was common. Overall, Tavo induced regression of at least one uninjected lesion in nearly half of patients, demonstrating clinical evidence of systemic anti-tumor immunity. In addition, a major benefit of the plasmid electroporation platform is that it can be modified relatively easily, based on translational data, to create next-generation therapies. Indeed, preclinical testing of a next-generation plasmid that induces expression of IL-12, CXCL9, and tumor membrane-anchored anti-CD3 is ongoing.

Intratumoral Tavo electroporation was well tolerated, and it did not induce the systemic symptoms associated with intravenous cytokine administration and even constitutional symptoms were mild and infrequent. No grade 4 adverse effects were noted, and only six patients had grade 3 adverse effects (local pain, five patients and cellulitis, one patient). While systemic cytokine administration induces fever, chills, and pyrexia suggesting a systemic inflammatory response, despite a high rate of regression of untreated lesions, these symptoms were not observed in patients treated with Tavo.

Intratumoral IL-12, as generated by Tavo, induces cDC1 and establishes DC-T cell crosstalk that mediates tumor rejection. Since cDC1 play a crucial role in recruiting and activating CD8+ T cells into the tumor microenvironment and are in turn induced by NK cells, we explored the effect of Tavo, in this publication, in a study by Garris et al., and in combination with PD-1 (manuscript submitted for review). Tavo induces activation across multiple classes of immune transcripts (Figure 4), including immune activation (Figure 4B), NK cell (Figure 4C), and antigen presentation (Figure 4D). The immune activation produced by Tavo results in both increased inflammatory gene expression, including expression of IFN-γ associated genes, and adaptive immune resistance, with increased expression of PD-L1 and TGFβ. This induction of adaptive resistance through PD-1/PD-L1 could explain the high proportion of responses to subsequent PD-1 blockade in patients progressing on Tavo (Figure 3B). Based on these findings, patients have now been treated on two prospective phase II clinical trials of Tavo in combination with the anti-PD-1 antibody pembrolizumab. A study of patients with few partially exhausted (PD1+CTLA-4+CD8+) cells has been completed and results will be reported elsewhere (submitted for publication) and a larger single-arm study in patients with documented progression on PD-1 blockade (KEYNOTE-695) is currently ongoing.

In summary, Tavo treatment drives changes in the immune microenvironment resulting in both local and global immune responses with minimal systemic toxicity. Our data demonstrate that this in situ tumor vaccination strategy can be a safe and effective approach to inducing multiple sustained, productive changes in the immune microenvironment that would be too toxic using similar systemic agents.

FUNDING

This study was supported by OncoSec Medical, Inc. (no grant number).

DISCLOSURE

AA is a paid advisor to OncoSec Medical, Inc. and he holds stock options in the company. He is also a paid advisor to Array, Regeneron, and Valitior. He also receives research funding from Acerta, Amgen, AstraZeneca, BMS, Dynavax, Genentech, Idera, Incyte, Idera, ISA, LOXO, Merck, Novartis, Regeneron, Sensei, and Tessa. He previously received research funding from Amgen, Celldex, GlaxosmithKline, Lilly, Medimmune, Plexicron, Roche, OncoSec Medical, Inc. SB, research funding from OncoSec Inc and Merck Inc. SA, research funding from OncoSec Inc and Merck Inc. KL receives research funding from OncoSec Inc and Merck Inc. MF receives research funding from OncoSec Inc; Advisor Boards of Novartis, Pulse Bioscience, Array Bioscience, Bristol Myers Squibb, Sanofi. LF receives research funding from OncoSec, Merck, AbbVie, Bavarian Nordic, BMS, Dendreon, Janssen, Roche/Genentech. CBB is advisor to PrimeVax, BMS. Stock ownership in BMS, Patent US20180322632A1: image processing systems and methods for displaying multiple images of a biological specimen. DB is an employee of OncoSec Medical, Inc. RT is an employee

8 https://doi.org/10.1016/j.annonc.2019.12.008
REFERENCES


