Poster: P524

Clinical and biomarker analysis of a phase II study of intratumoral tavokinogene telseplasmid (pIL-12) plus pembrolizumab in stage III/IV melanoma patients predicted to not respond to anti-PD-1

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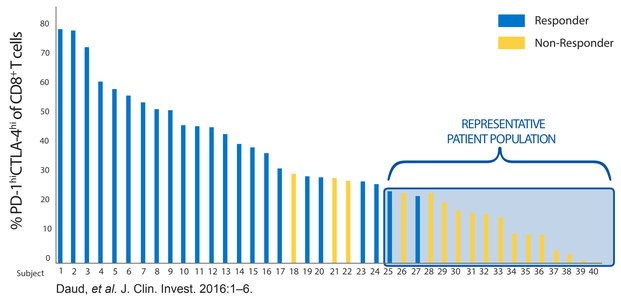
BACKGROUND

- Clinical success of anti-PD-1/PD-L1 immunotherapies is limited to a minority of patients with solid tumors. Patients with immunologically cold tumors are characterized by a low frequency of tumor infiltrating lymphocytes (TIL) and tend to not respond to checkpoint therapeutics.
- OncoSec's intratumoral delivery of plasmid IL-12 (tavokinogene telseplasmid; tavo) by electroporation (IT-tavo-EP) can reshape the tumor microenvironment (TME), transforming both treated and untreated lesions into CD8$^+$T cell inflamed tumors (ASCO-SITC 2017).
- Updated clinical (locked September 12th, 2017) and biomarker analyses from OncoSec's combination trial with IT-tavo-EP and pembrolizumab in patients predicted to not respond to anti PD-1/PD-L1 monotherapies are presented here.

STUDY OVERVIEW

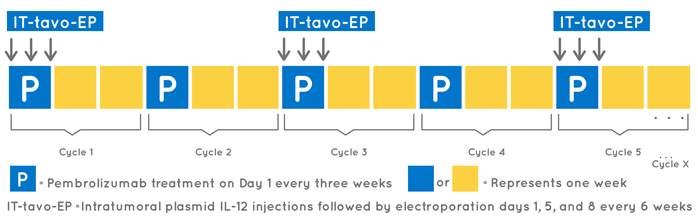
PATIENT SELECTION



Daud, *et al.* J. Clin. Invest. 2016:1–6.

Flow cytometric analysis of patient tumor biopsies taken pre-treatment. Patients with CD8$^+$ TIL with < 25% PD-1^{hi}CTLA-4^{hi} are highly unlikely to respond to anti-PD-1 therapies and are representative of the patients enrolled in this trial.

STUDY DESIGN



Patients must have histological or cytological diagnosis of melanoma with locally advanced or metastatic disease that is not amenable to definitive local therapy with curative intent and at least one measurable tumor accessible for intratumoral injection. Pembrolizumab was administered as a 30 minute intravenous infusion at a dose of 200 mg every 3 weeks with intratumoral IT-tavo-EP on Days 1, 5, and 8 of every odd cycle (6 week intervals).

PRIMARY OBJECTIVE: Measure the best overall response rate (BORR) by RECIST v1.1 of combination IT-tavo-EP and pembrolizumab in this low PD-1^{hi}CTLA-4^{hi}TIL cohort.

BIOMARKER STUDIES: Patient pre- and post-treatment tumor biopsies and blood were collected and analyzed for various immunological endpoints.

MATERIALS AND METHODS

RNA ANALYSIS: Total RNA isolated from formalin-fixed paraffin embedded (FFPE) tumor biopsies collected from patients pre- or post-treatment were analyzed using the PanCancer IO 360™ beta version or Human Immunology v2 Gene Expression Panels (NanoString® Technologies). Raw mRNA abundance frequencies were analyzed using nSolver analysis software 3.0 pack.

TCRβ SEQUENCING: DNA isolated from FFPE tumor biopsies and peripheral blood mononuclear cells (PBMC) of patients pre- and post-treatment were used in immunoSEQ T cell Receptor (TCR) assays (Adaptive Biotechnologies®).

FLOW CYTOMETRY: PBMC isolated from patient blood pre- or post-treatment were stained for cell surface markers before acquisition on a LSR Fortessa X-20 flow cytometer (BD Biosciences). Cell populations were analyzed for immune phenotype using FlowJo Software (BD Biosciences). Cells were gated on single, live, CD3$^+$ populations.

IMMUNOHISTOCHEMISTRY: FFPE tumor biopsies were stained for immune markers and analyzed by multispectral imaging (Fox Lab) in a blinded fashion. Digital images were captured with PerkinElmer Vectra platform. Regions of interest with the highest immune cell infiltrates were scanned at 20X and selected for analysis. Three images of 0.36 mm^2 each were analyzed per sample with InForm Software (PerkinElmer).

ACKNOWLEDGEMENTS

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Contact Dr. Alain Algazi with any questions: Alain.Algazi@ucsf.edu

Trial Registration - NCT02493361

The NanoString® assays and instrumentation used to generate results in this study are for Research Use Only, not for use in diagnostic procedures.

CLINICAL DATA

DEMOGRAPHICS

Age (years)	
N / mean (SD)	23 / 65.5 (12.2)
Median, min max	67, 39, 91
Sex - Male / Female	13 (56.5%) / 10 (43.5%)
Stage at enrollment	
Stage IIIb and IIIc	14 (60.9%)
Stage IV M1a and M1b	4 (17.4%)
Stage IV M1c	5 (21.7%)
Prior checkpoint inhibitors	
anti-PD-1 only	4 (17.4%)
anti-CTLA-4 only	1 (4.3%)
anti-PD-L1 and anti-CTLA-4	6 (26.1%)
BRAF	
Mutant	7 (30.4%)
Unknown, Wild type	16 (69.6%)

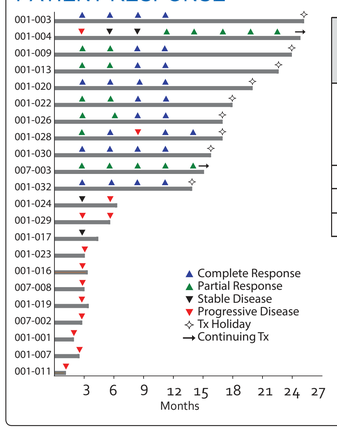
SAFETY

Reporting of treatment-emergent adverse events (TEAE) grade 3 or greater. All issues were resolved and patients recovered.

Patient	Adverse Event	Related to pembro	Related to IT-tavo-EP	Grade	SAE
001-004	PAIN DUE TO EP	UNRELATED	DEFINITELY	3	
	CHILLS	POSSIBLE	POSSIBLE	3	
	COLD SWEAT	POSSIBLE	POSSIBLE	3	
	NON-ST-ELEVATION MYOCARDIAL INFARCTION (NSTEMI)	UNLIKELY	UNLIKELY	3	SAE
001-013	ELEVATED LFT	UNRELATED	UNRELATED	3	
	BILIARY COLIC	UNRELATED	UNRELATED	3	
001-019	CELLULITIS	UNLIKELY	POSSIBLE	3	SAE
007-002	ANEMIA	UNRELATED	UNRELATED	3	

pembro = pembrolizumab

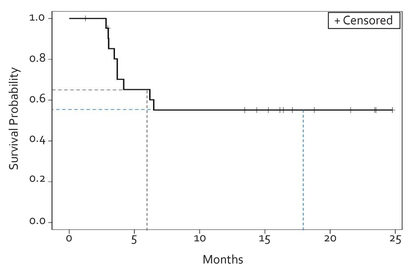
PATIENT RESPONSE



BORR on Combo Therapy	Patient	Previous Immunotherapies in Responding Patients			
		Medication	Cycles	Dose	Best Response
PR	001-004	Ipilimumab	2	Unknown	PD
		Pembrolizumab	1	Unknown	Unknown; AE - severe headache
		Nivolumab	4	1 mg/kg	PD
CR	001-013	Pembrolizumab	1	200 mg	Unknown
CR	001-028	TVEC	3	1 mL	PD
PR	007-003	Ipilimumab	4	3 mg/kg	PD

Best Overall Response Rate (BORR = CR + PR)	11 (50%)
Disease Control Rate (DCR = CR + PR + SD)	13 (59%)
Complete Response (CR)	9 (41%)
Partial Response (PR)	2 (9%)
Stable Disease (SD)	2 (9%)
Progressive Disease (PD)	9 (41%)

PROGRESSION FREE SURVIVAL (PFS)

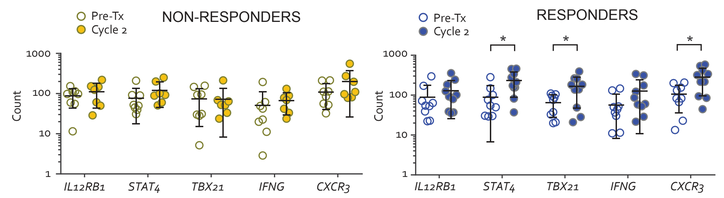


Clinical response at 15 months:
- Best overall response rate (BORR) 50% (11/22)
- Duration of response (DOR) 100% (11/11)
- Progression Free Survival (PFS) 57%
- Disease Control Rate (DCR) 59% (13/22)

Response Type	N
RECIST v 1.1	9
Progression then response	1
Non-measurable (< 1 cm)	1

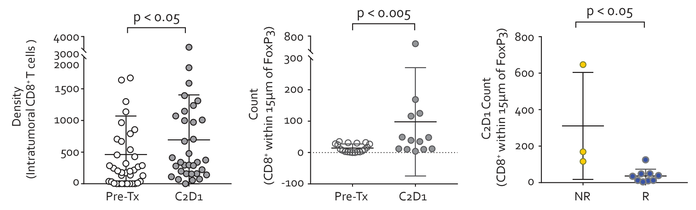
BIOMARKER DATA: ANALYSIS OF THE TUMOR MICROENVIRONMENT (TME)

NANOSTRING ANALYSIS OF FFPE TUMOR BIOPSIES



Responding patients had a significant increase of intratumoral expression of Th1-related genes such as *STAT4, TBX21* (T-bet) and *CXCR3* after 1 cycle of treatment with the combination of IT-tavo-EP and pembrolizumab. *$p \leq 0.05$

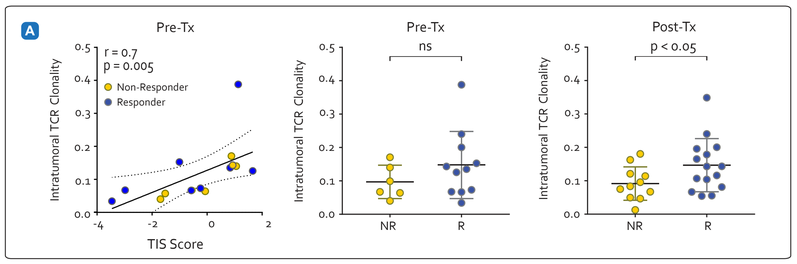
IHC ANALYSIS OF FFPE TUMOR BIOPSIES



mIHC analysis of FFPE tumor biopsies revealed a significant increase in intratumoral CD8$^+$ T cells after a single cycle of combination treatment. Spatial analysis showed a significant increase in the number of FoxP3$^+$cells < 15 µm from CD8$^+$T cells in patients after a single cycle of treatment. Post-treatment, non-responders showed a significantly higher amount of FoxP3$^+$ cells < 15 µm from CD8$^+$T cells when compared to responders.

BIOMARKER DATA: RELATIONSHIP OF TME AND PERIPHERAL RESPONSE

TCRβ SEQUENCING OF FFPE TUMOR BIOPSIES AND BLOOD



(A) Tumor biopsies of enrolled patients pre-treatment had low TCR clonality that correlates with the Tumor Inflammation Signature (TIS) score (NanoString® Technologies). Combination IT-tavo-EP and pembrolizumab therapy increased intratumoral TCR clonality in responding patients after treatment compared to non-responders. (B) Responding patients had a significant post-treatment increase of intratumoral TCR clonality compared to PBMC in the periphery. (C) Post-treatment TCR clonality in tumors correlated strongly with the treatment-related increase of proliferating PD1$^+$TIGIT$^+$CD8$^+$ T cells. These exhausted T cells were significantly elevated in the periphery of patients after treatment. Only responding patients had a significant increase in circulating PD-1$^+$TIGIT$^+$ T cells.

**Untreated tumors*

SUMMARY

Clinical data suggests combination IT-tavo-EP continues to be an effective therapeutic modality in patients unlikely to respond to anti-PD-1 therapies. We demonstrate:

- Best overall response rate (BORR) of 50% (11/22); (43% [9/21] achieved RECIST v1.1 BORR)
  - Complete response (CR) rate of 41% (9/22); (38% [8/21] achieved RECIST v1.1 durable CR)
- Disease control rate (DCR) of 59% (13/22); (52% [11/21] achieved RECIST v1.1 DCR)
- Progression free survival (PFS) of 57% at 15 months
- Duration of response (DOR) of 100% (11/11)

Associated biomarker data highlights connected immunological mechanisms that positively impact this patient population:

- Responding patients had a significant treatment-related increase in the density of intratumoral CD8$^+$ T cells coupled with a significant increase of intratumoral Th1-related gene expression
- Spatial analysis revealed a significant post-treatment increase of FoxP3$^+$ cells < 15 µm from CD8$^+$ T cells in non-responding patients
- Tumor biopsies from pre-treatment timepoints had a low overall TIS score (average score of -0.37) as expected, yet still positively correlated with intratumoral TCR clonality
- Intratumoral TCR clonality significantly increased after a single cycle of treatment
- Responders had low peripheral TCR clonality with significantly higher intratumoral TCR clonality after treatment but in non-responders, this relationship was inverted with no significance noted
- Proliferating exhausted peripheral CD8$^+$ T cells significantly correlated with intratumoral TCR clonality
- The percentage of exhausted CD8$^+$ T cells significantly increased after combination treatment, with only responding patients maintaining a significant increase in total proliferating exhausted T cells

CONCLUSIONS

Durable clinical responses continue to demonstrate that this combination therapy is a promising treatment modality. The updated correlative immune-focused biomarker data further highlights that this IL-12-based therapy can drive intratumoral Th1 polarization and an increase of CD8$^+$ TIL with less spatial proximity to suppressive FoxP3$^+$ cells in responding patients. Additionally, increased treatment-related intratumoral TCR clonality and proliferating exhausted T cells in the periphery of responding patients extends the concept that IT-tavo-EP and pembrolizumab combination therapy can reshape the TME. Collectively, these data suggest combination IT-tavo-EP and pembrolizumab therapy directs the TME towards a Th1 immune phenotype with reduced immune suppression, demonstrating robust intratumoral and systemic anti-tumor responses, which support improved clinical outcomes in patients predicted not to respond to anti-PD-1 therapy.