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

Alain P., Algazi J., Katy K. Tsai1, Michael D. Rosenblum, Robert Andtbacka, Carmen Ballesteros-Merino, Shawn Jensen, Carlo B. Bifulco1, Bernard A. Fox, SuFey Ong, Alessandra Cesano, Joseph Beechem, Chris Twitty, Jean S. Campbell, Victoria Shainsky, Donna Bannavong, Erica Browning, Reneta Talia, Shawna Shirley, Mai H. Le, Robert H. Pierce, Sharron Gargosky, Adi I. Daud*

1University of California, San Francisco Medical Center. Mt. Zion, 1600 Divisadero Street, San Francisco, CA 94115; Huntsman Cancer Institute, 1550 Circle of Hope Drive, Salt Lake City, Utah 84142; *Eagle A. Chiles Research Institute at Providence Portland Medical Center, Portland, Oregon 97213; 2NanoString Technologies, 500 Fairview Avenue N, Seattle WA 98109; 3Oncole use Medical Incorporated, 5820 Nacy Ridge Drive San Diego CA 92121

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 therapies.
- Oncosec’s intratumoral delivery of plasmid IL-12 (tavokinogene telseplasmid; tavo) by electroporation (IT-tavo-EP) can reestablish the tumor microenvironment (TME), transforming both treated and untreated lesions into CD8+ T-cell infiltrated tumors (ASCO-GTC 2017).
- Updated clinical data (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

Flow cytometry analysis of patient tumor biopsies taken pre-treatment. Patients with CD8+ TIL with < 25% PD-1/PD-L1+ are highly unlikely to respond to anti-PD-1 therapies and are representative of the patients enrolled in this trial.

STUDY DESIGN

Treatment groups:
- IT-tavo-EP: Tumor biopsies taken pre-treatment and blood were collected and analyzed for various immunological endpoints.
- TCR Clonality: Intratumoral TCR clonality significantly increased after a single cycle of treatment.
- Flow Cytometry: Intratumoral TCR clonality significantly increased after combination treatment, with only responding patients maintaining a significant increase in total proliferating exhausted T cells.

Materials and Methods
- RNA Analysis: Total RNA was isolated from formalin-fixed paraffin embedded (FFPE) tumor biopsies collected from patients pre- or post-treatment using the PanCancer ID 360™ beta version or Human Immunology Panel v2 Gene Expression Panels (NanoString Technologies). Raw mRNA abundance frequencies were analyzed using nSolver analysis software 3.0 pack.
- TCR Sequencing: DNA isolated from FFPE tumor biopsies and peripheral blood mononuclear cells (PBMC) of patients pre- and post-treatment were used in immuneSEQ™ 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 cytometry (BD Biosciences). Cell populations were analyzed for immune phenotype using FlowJo software (BD Biosciences). Cells were gated on live, CD3+ populations.
- Immunohistochemistry: FFPE tumor biopsies were stained for immune markers and analyzed by multiplexed imaging (Fox Lab) in a blinded fashion. Digital images were captured using PerlinImmer Vectora platform. Regions of interest with the highest immune cell infiltrates were scanned at 20X and selected for analysis. Three images of 0.36 mm² each were analyzed per sample with Inform Software (PerlinImmer).

Acknowledgements
We would like to gratefully thank the patients and their families for supporting this trial. We thank Mierk for providing pembrolizumab and Oncosec for the IT-tavo-EP.

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

Total Registration: NCT02493361

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

Clinical response at 15 months:
- Best overall response rate (BORR) 50% (11/22)
- Complete response (CR) rate of 41% (9/22)
- Progression free survival (PFS) 57%
- Disease Control Rate (DCR) 93% (19/22)

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 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 TSS 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 lower 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 cumulative 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 extend the concept that IT-tavo-EP and pembrolizumab combination therapy can reestablish 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.