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

Alain P. Algozzi 1, Katy K. Tsai 1, Michael D. Rosenblum 2, Robert Andtbacka 3, Carmen Ballesteros-Merino 4,4, Shawn Jensen 4, Carlo B. Bifulco 5, Bernard A. Fox 5, SuFey Ong 6, Alessandra Cesano 7, Joseph Beechem 8, Chris Twitty 9, Jean S. Campbell 10, Victoria Shainsky 11, Donna Bannavong 12, Erica Browning 13, Reneta Tallia 13, Shawn Shirley 14, Mai H. Le 15, Robert H. Pierce 16, Sharron Gargosky 17, Adi I. Daud 18

1University of California, San Francisco Medical Center-Mt. Zion, 1600 Divisadero Street, San Francisco, CA 94115, 2Huntsman Cancer Institute, 1950 Circle of Hope Drive, Salt Lake City, Utah 84142, 3Eal: E. Chiles Research Institute at Providence Portland Medical Center, Portland, Oregon 97213, 4NanoString Technologies, 500 Fairview Avenue N, Seattle WA 98109, 6Oncosec Medical Incorporated, 5820 Nancy 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 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-2017).

• Updated clinical locked (9/12/2017) 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 cytometric 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

IT-tavo-EP combination treatment. Spatial analysis showed a significantly higher amount of FoxP3+ T cells when compared to responders.

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 T1 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.

BIOMARKER DATA: RELATIONSHIP OF TME AND PERIPHERAL RESPONSE

TCRβ SEQUENCING OF FFPE TUMOR BIOPSIES AND BLOOD

- Complete response (CR) rate of 41% (9/22);[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 < 15μm from CD8+ T cells in non-responding patients
- Tumor biopsies from pre-treatment timepoints had a low overall TIL score (average score of -0.37) as compared to post-treatment biopsies. The increased TCR T cells significantly increased after combination treatment, with a significant increase in circulating PD-1+ T cells.
- 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
- 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% at 15 months
  - Disease control rate (DCR) 59% (13/22)

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 STAT3, TLR4 (T322) and CXCL9 after 1 cycle of treatment with the combination IT-tavo-EP and pembrolizumab.

IHC ANALYSIS OF FFPE TUMOR BIOPSSIES

- mRN 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 < 15μm from CD8+ T cells in patients after a single cycle of treatment. Post-treatment, non-responders showed a significantly higher amount of FoxP3+ cells < 15μm from CD8+ T cells when compared to responders.

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 Progenika (iQ) RT-PCR on Human Immunology v2 Gene Expression Panels (NanoString Technologies). Raw mRNA abundance frequencies were analyzed using iSAGE software version 3.0.3 pack.

TCRβ SEQUENCING: DNA isolated from FFPE tumor biopsies and peripheral blood mononuclear cells (PBMC) of patients pre- or post-treatment were used in immuneSeqT cell Receptor (TCR) assay (Adaptive Biotechnologies). Flow Cytometry: PBMC isolated from patient blood pre- or post-treatment were stained for cell surface markers before acquisition on a LSRFortessa® X-20 flow cytometry (BD Biosciences). Cell populations were analyzed for immune phenotype using FlowSage Software (BD Biosciences). Cells were gated on single, live, CD3+ populations.

IMMUNOHEMATOLOGY: FFPE tumor biopsy sections were stained for immune markers and analyzed by multiphoton 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 mm² each were analyzed per sample with InForm Software (PerkinElmer).

ACKNOWLEDGEMENTS

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

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

Total Registration: NCT02493361

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