AN INTRATUMORAL TREATMENT MODALITY TO ADDRESS AN UNMET MEDICAL NEED: RESULTS FROM A PHASE II COMBINATION STUDY WITH pIL-12 PLUS PEMBROLIZUMAB IN MELANOMA PATIENTS PREDICTED TO NOT RESPOND TO ANTI-PD-1 THERAPY

Adapted from ASCO-SITC Poster No. 78

Alain Algazi¹, Katy Tsai¹, Michael Rosenblum¹, Prachi Nandoskar¹, Robert H.I. Andtbacka², Amy Li¹, Toshimi Takamura³, Mary Dwyer³, Erica Browning³, Reneta Talia³, Chris Twitty³, Sharron Gargosky³, Jean Campbell³, Carmen Ballesteros-Merino⁴, Carlo B. Bifulco⁴, Bernard A. Fox⁴, Mai H. Le³, Robert H. Pierce³, Adil Daud¹

¹ University of California, San Francisco Medical Center-Mt. Zion, 1600 Divisadero St, San Francisco, CA 94115, ² Huntsman Cancer Institute, 1950 Circle of Hope Drive, Salt Lake City, Utah 84112, ³ Oncosec Medical Inc., 5820 Nancy Ridge Drive San Diego CA 92121, ⁴ Earle A. Chiles Research Institute at Providence Portland Medical Center, Portland, Oregon 97213
**BACKGROUND**

Recent publications support the emergence of predictive biomarkers for pembrolizumab in melanoma based on the expression of PD-L1 in the tumor microenvironment (Daud, et al. *Journal of Clinical Oncology*. 2016:1–12) or the frequency of PD-1<sup>hi</sup>CTLA-4<sup>hi</sup> on CD8<sup>+</sup> tumor infiltrating lymphocytes (TIL) (Daud, et al. *J. Clin. Invest*. 2016:1–6), whereby immunologically inactive tumors are associated with a poor response. Since intratumoral electroporation of plasmid IL-12 (IT-pIL12-EP) increases TIL in both treated and untreated lesions, we hypothesize that a poor response with anti-PD-1 monotherapy can be reversed with the combination of IT-pIL12-EP and anti-PD-1.

**DESIGN**

Metastatic and unresectable melanoma patients with accessible lesions were enrolled. Only patients who were considered to be unlikely to respond to anti-PD-1 therapy based on a frequency of CD8<sup>+</sup>CD45<sup>+</sup> TIL that are <25% PD-1<sup>hi</sup>CTLA-4<sup>hi</sup> by flow cytometric analysis of a fresh tissue biopsy (see below) were eligible to receive treatment on study. Eligible patients were treated with IT-pIL12-EP in accessible lesions on Days 1, 5 and 8 every six weeks (cycles 1, 3, 5, 7 and 9) and with intravenous pembrolizumab (200 mg) on Day 1 of each 3-week cycle.

**Primary Objective:**
To assess the anti-tumor efficacy (defined as the best overall response rate using RECIST v1.1) of the combination of intratumoral pIL-12 EP and pembrolizumab in patients with low TIL melanoma.

**Secondary Objectives:**
To assess safety and tolerability of the combination of intratumoral pIL-12 EP and pembrolizumab; duration of response; twenty-four week landmark progression free survival; median progression free survival; overall survival; and best overall objective response rate by immune related-Response Criteria (irRC) in “low TIL” melanoma patients treated with the combination of intratumoral pIL-12 EP and pembrolizumab.

**Biomarker Studies:**
Patients’ pre- and post-treatment blood and tumor biopsy specimens were collected for various biomarker endpoints.

**FIGURE 1** Patient Selection

(A) Recent publications by Daud, et al. highlight the use of an IHC-based or flow cytometric-based assay to predict patients’ responses to pembrolizumab.
B Enrolling patients unlikely to respond to anti-PD-1

These assays were used to interrogate patients’ tumor biopsies taken during screening. Analysis of biopsies with both the TIL flow cytometry and the melanoma-specific IHC assays demonstrated a correlation between the two endpoints. Additionally, patients enrolled on the trial had tumors with less evidence of adaptive resistance compared to excluded patients.

FIGURE 2 Study Overview

A Study Design

An overview of the “Phase II, Multicenter Study of Enhancing Pembrolizumab Responses in Melanoma through Intratumoral pIL-12 Electroporation” clinical trial (NCT02493361). (A) Study design noting the standard 3 week intervals of 200 mg pembrolizumab and the 6 week intervals of intratumoral pIL-12 electroporation treatment cycles.

B Overview of ImmunoPulse® IL-12 (IT-pIL12-EP)

(B) An illustration to highlight the general mechanisms of ImmunoPulse® IL-12 therapy.
**FIGURE 3 Clinical Data**

**A Swimmer’s Plot**

- Complete Response
- Partial Response
- Stable Disease
- Progressive Disease
- Tx Holiday
- Continuing Tx

* Previous vs. baseline
** Baseline not measurable per RECIST <1cm

**B Response Table**

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**Safety:** Treatment was well tolerated; 38% of adverse events (AE) were classified as treatment site reactions (grade 1-2) that resolved. One reported SAE of Grade-3 cellulitis resolved with 5d antibiotics. One reported Grade-2 ECI of diarrhea resolved with corticosteroids.

**Response:** Overall response rate (ORR) evaluated using RECIST v1.1 by investigator evaluation at each re-staging assessment performed approximately every 12 weeks showed a 43% ORR at 24 weeks and overall best ORR of 48%

**Demographics:** These 22 patients, ages 39-89 years, were 53% male, 66% Stage III and 34% Stage IV

**FIGURE 4 Reshaping of the Tumor Microenvironment**

**A Productive intratumoral immune responses by NanoString analysis**

(A) NanoString analysis of mRNA isolated from FFPE tumor biopsies at screening and after 1 cycle of treatment demonstrates increased adaptive resistance and gene expression associated with productive anti-tumor immune responses in the responding patients.

The combination IT-pIL12-EP with pembrolizumab transforms poorly infiltrated/non-inflamed tumors into immunologically active lesions.
FIGURE 4 Reshaping of the Tumor Microenvironment (continued)

A Productive intratumoral immune responses by NanoString analysis

B Multispectral IHC analysis of FFPE tumor biopsies

(C) Adaptive Biotechnologies TCRβ sequencing analysis also suggests that combination therapy increases TIL frequencies in poorly infiltrated tumors. In addition to increased TIL, a notable correlation with T cell clonality was established in responding patients' tumors.

(B) Multispectral IHC (mIHC) analysis of tumor biopsies highlights the changes in immunological activity at the tumor site and how the ratios of CD8+ TIL to total or CD163+ restricted expressing PD-L1+ cells relate to clinical responses (NR = non-responder (SD/PD); R = responder (PR/CR)).
Intratumoral electroporation of pIL-12 with anti-PD-1 can trigger productive peripheral immune responses, as well as increase TIL and regress untreated (non-electroporated) lesions. (A) mIHC analysis of tumor biopsies illustrates an increase in TIL relative to screening in non-electroporated lesions, regardless of response. The relative ratio of CD8:PD-L1 cells is consistent with response in both EP and non-EP lesions.

(B) PET scan of a responding patient at screen and week 12 (cycle 4) points to the regression of an untreated lesion in the inguinal lymph node.
Antigen-specific immune response with IFN-γ ELISpot

(D) Tumor antigen-specific functional immune responses from PBMC of a healthy donor, a responding and a non-responding patient were assessed by IFN-γ ELISpot (n = 2 in 2 independent experiments)

CONCLUSION

The excellent safety profile and striking 43% clinical response rate at 24 weeks (BORR of 48%) is encouraging as treated patients were predicted to have appreciably lower responses by published predictive biomarker assays. The correlative biomarker data draws from varied immune-focused endpoints addressing gene expression and multispectral IHC in the tumor microenvironment, changes in clonality by TCRβ sequencing and peripheral immune phenotype and function (flow cytometry and ELISpot, respectively) which broadly supports an immune-directed mechanism that is differentiated between responders and non-responders.

Collectively, this data suggests that IT-pIL12-EP plus pembrolizumab can effectively alter the tumor microenvironment by triggering adaptive resistance through the IL-12/IFN-γ axis, increasing the substrate for a therapeutic PD-1/PD-L1 blockade while driving systemic anti-tumor immunity and concordant clinical responses in patients unlikely to benefit from anti-PD-1 monotherapy.

Based on these interim clinical responses and biomarker data, a registration-enabling phase II clinical trial with IT-pIL12-EP plus pembrolizumab is planned to enroll in 2017 to treat patients with melanoma that are progressing on either pembrolizumab or nivolumab treatment.

Contact Dr. Alain Algazi with any questions:
Alain.Algazi@ucsf.edu
We would like to gratefully thank the patients and their families, as well as Merck, for supporting this trial with pembrolizumab, and Oncosec Medical Incorporated, for providing support and the IT-pIL12-EP.