Systemic anti-tumor effect and clinical response in a phase 2 trial of intratumoral electroporation of plasmid interleukin-12 in patients with advanced melanoma.

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ABSTRACT

Background: Interleukin-12 (IL-12) promotes anti-tumor activity through multiple mechanisms, including augmentation of adaptive and innate immune responses. Intratumoral (IT) delivery of IL-12 via electroporation (EP) avoids systemic toxicity while promoting systemic antitumor immunity. This phase 2 study explores the systemic efficacy, clinical response and safety of IT plasmid IL-12 injection (pIL-12) followed by EP in patients (pts) with advanced melanoma.

Methods: This single-arm, open-label phase 2 study plans to enroll 30 pts with in-transit or M1a melanoma. One treatment cycle consists of IT pIL-12-EP on days 1, 5, 8 in up to four lesions per cycle. A maximum of four cycles at 12-week intervals are allowed. ORR was assessed by a modification of RECIST for cutaneous lesions with restaging performed every 12 weeks. The primary endpoint is best ORR within 24 weeks of first treatment. Pre- and post-treatment tumor biopsies were obtained in all patients. Ongoing analyses to assess safety and emerging efficacy data are being utilized to inform future studies.

Results: 30 pts have been enrolled and have received at least one treatment cycle. The ORR is 32% (9/28), with 10.7% CR (3/28). Twenty-two pts had evaluable non-injected lesions, with 13/22 (59.1%) showing at least one non-injected lesion regressing from baseline. Pain (69.0%) and inflammation (20.7%) at the treatment site were the most common grade 1/2 drug-related adverse events (AEs). One grade 3 AE of pain with electroporation was reported. No serious adverse events (SAEs) related to treatment have been reported. Exploratory analyses indicate a doubling of intratumoral NK cells from pre-treatment through day 11 and at day 39, and increased frequency in activated circulating NK cells.

Conclusions: Local treatment with pIL-12-EP is well tolerated without severe systemic side effects. Regression of treated and non-treated tumors suggests successful induction of systemic anti-tumor response. Local and systemic increases in NK cells are consistent with the expected pharmacodynamic effect of IL-12. Based on these data, an expansion protocol to evaluate increased treatment frequency is planned for melanoma patients.

INTRODUCTION

Plasmid Interleukin-12 (pIL-12)

- Nonviral, high copy number plasmid designed to achieve transient transfection of the host cell and high level of IL-12 protein expression

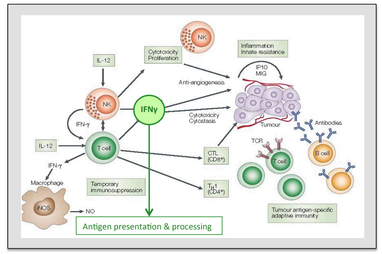
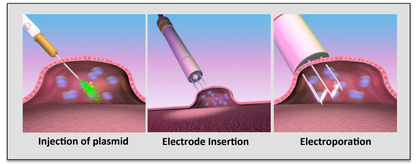


Fig. 1. Proposed anti-tumor activity of IL-12. ***Adapted from Trinchieri-G, Nature Reviews Immunol V3 FEB2003***

Electroporation (EP)

- Transmembrane potential generated via brief, controlled electrical pulses at a specified electrical voltage

Fig. 2. *In-vivo* Electroporation



STUDY OBJECTIVES

Primary:

- To assess the objective response rate at 24 weeks

Secondary:

- To assess the distant response rate of untreated lesions
- To assess the local response rate of treated lesions
- To describe the immunologic effects of pIL-12 EP

STUDY DESIGN

Population

- Enrollment of 30 patients with stage IIIB-IV melanoma with cutaneous or intransit lesions accessible to electroporation

Treatment Regimen

- Treatment days 1, 5 and 8 of a 90 day cycle

Efficacy

- Modification of RECIST for cutaneous lesions with restaging performed every 12 weeks. Modifications include:
  - Clinically measurable lesions of at least 0.3 cm in longest diameter considered target lesions
  - New lesions not an indicator of PD but added to the disease burden as target lesions

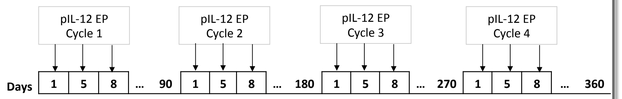


Fig. 3. Treatment regimen demonstrating four treatment cycles over one year. Each cycle consisted of three treatment days (Days 1, 5 and 8). Disease evaluations were performed every twelve weeks (Days 90, 180, 270 and 360.

PATIENT DEMOGRAPHICS

GENDER	**Male**	16/30 (53.3%)	**Female**	14/30 (46.7%)		
AGE	**Median**	67 years	**Min-Max**	49-88 years		
RACE	**Caucasian**	27/30 (90.0%)	**Asian**	1 /30(3.3%)	**Other (Unknown)**	2/30 (6.7%)
ECOG	**0**	21/30 (70.0%)	**1**	9/30 (30.0%)		
STAGE	**IIIB**	6/30 (20.0%)	**IIIC**	13/30 (43.3%)	**IV**	11/30 (36.7%)

SAFETY RESULTS

Grade 1/2 Adverse Event (Possible or definitely related)	Patients Reported (%)
Pain	20 (69.0%)
Inflammation	6 (20.7%)
Fatigue	5 (17.2%)
Soreness at treatment site	4 (13.8%)
Bruising at treatment site	3 (10.3%)
Discoloration at treatment site	3 (10.3%)
Rash	3 (10.3%)
Pruritus	3 (10.3%)
Erythema	2 (6.9%)
Cellulitis	2 (6.9%)
Discharge at treatment site	2 (6.9%)

- No SAEs related to treatment have been reported; one patient report of Grade 3 pain with electroporation

CLINICAL RESULTS

BEST ORR by modified RECIST

Number of evaluable patients (patients to reach primary endpoint)	Objective response (%)	Complete response (%)
28	9/28 (32.1%)	3/28 (10.7%)

SYSTEMIC RESPONSE by modified RECIST

Number of evaluable patients (patients with untreated lesion(s) from baseline)	Patients with regression of at least one non-injected lesion (%)
22	13/22 (59.1%)

TREATED LESION BEST RESPONSE by modified RECIST

Number of treated lesions evaluated	Stable (%)	Partial response (%)	Complete response (%)
85	26/85 (30.6%)	7/85 (8.2%)	38/85 (44.7%)

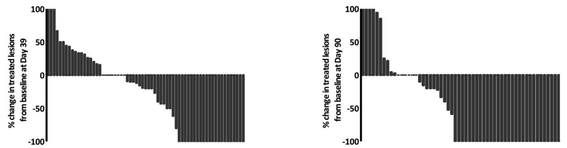


Fig. 4. Percent change of longest diameter in treated lesions at Day 39 (N=68) and Day 90 (N=57).

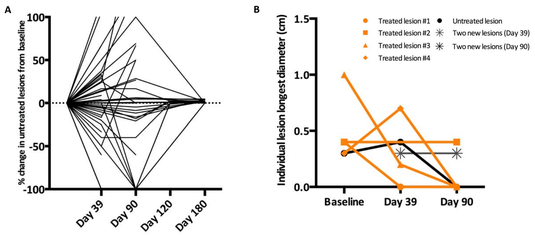


Fig. 5. A) Percent change of longest diameter in untreated lesions over time B) Lesion plot depicting size of individual lesions for Subject 001-019. Four lesions were treated during Cycle 1 (depicted in orange). Complete response in three of four and stabilization in one of four treated lesions seen by Day 90. New lesions at Day 90 would undergo treatment in Cycle 2.

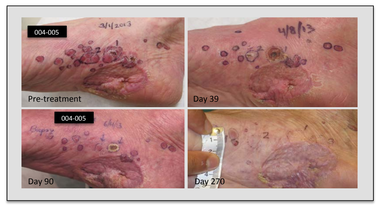


Fig. 6. Clinical photos of response over time in Subject 004-005. Lesions 1 and 2 photographed at pre-treatment were treated during Cycle 1.

CORRELATIVE RESULTS

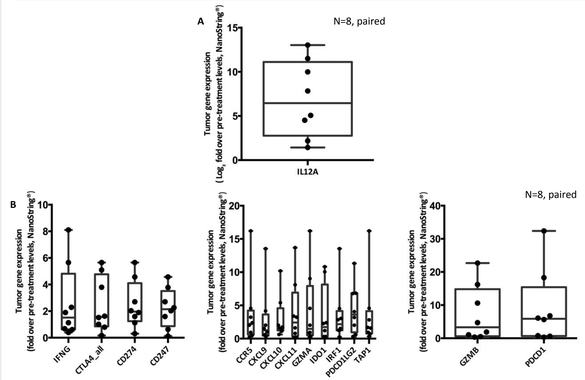


Fig. 7. Direct measures of gene expression in treated tumors assessed by NanoString® nCounter technology in pre- and Day 11 (3 days post-treatment) paired samples; A) Log_2 fold change over baseline of IL12A expression in 8 paired pre- and Day 11 (3 days post-treatment) tissue from treated lesions at B) Fold changes over baseline of IFNγ-inducible genes in 8 paired pre- and Day 11 (3 days post-treatment) tissue from treated lesions.

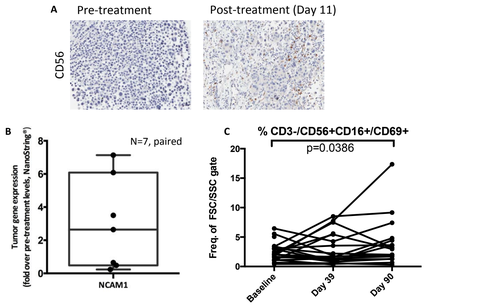


Fig. 8. Changes in intratumoral and circulating NK cells was assessed in paired pre- and post-treatment samples; A) Intratumoral change in NK cells in treated tumors was assessed by IHC for CD56 in pre- and Day 11 (3 days post-treatment) paired samples B) Fold change over pre-treatment levels of NCAM1 in 7 paired pre- and Day 11 (3 days post-treatment) tissue by NanoString® nCounter technology supported increased gene expression C) increased frequency in activated circulating NK cells observed by flow cytometry post-treatment.

CONCLUSIONS

- Local treatment with pIL-12 EP is well tolerated without severe systemic side effects
- Regression of non-treated tumors suggests successful induction of systemic anti-tumor response
- Increases in IL-12, IFNγ inducible genes and NK cells are consistent with pharmacodynamic effect of IL-12
- Data from this Phase 2 study warrants further evaluation of pIL-12 EP
  - An expansion protocol to evaluate increased dose intensity will be initiated
  - A randomized Phase 2b study is planned