First-in-human (FIH) trial evaluating immune activation and safety of PIN- 2 administered IV to patients with advanced solid tumors.

Abstract #2600

Colin Bier[1*], Michael Millward[2], Dusan Kotasek[3], Kenneth J. Gorelick[1], Joshua Brian Goldberg[1]

[1]PIN Pharma, Inc. New York, NY; [2]School of Medicine and Pharmacology, Nedlands, Australia; [3]Adelaide Cancer Center and University of Adelaide, Kurralta Park, Australia; *Presenting Author

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Introduction

Progressive immunosuppression is concomitant with tumor progression. Development of effective cancer immunotherapies for patients with advanced solid tumors has been clinically challenging in light of the associated adverse effects and low response rates of approved immunotherapies.

Currently approved Immuno-Oncology strategies have primarily focused on T-cell activity to illicit antitumor immunity. Antitumor immune responses involve systemic coordination across various cell types and tissues. Activating cells of the innate immune lineage in peripheral blood with immunostimulatory agents represents a promising strategy for global anticancer immunity in patients with advanced tumor burden.

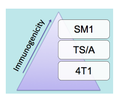
Enhancement of peripheral innate immune signaling to link adaptive immunity is an effective strategy to promote antitumor immune responses that may improve patient response rates.

PIN-2 is a novel immunomodulatory agent derivatized from a transactivator (Tat) protein that enhances innate immunity by stimulating differentiation of monocyte precursors to promote maturation of antigen presenting cell (APC) to dendritic cells (DC) that coordinate the adaptive immune response.

A first-in-human trial (#ACTRN12617001597381) was conducted to evaluate the safety, tolerability, and treatment-associated induction of immune activation by PIN-2 (signal) in all comers with advanced solid tumors.

Background

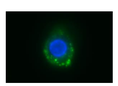
- *PINS (Precision Immune Stimulants)* drive the DC-APC innate immunity complex to stimulate endogenous T-cell mediated immune responses (linking adaptive immunity).



- Transactivator (Tat) protein swap derivatives between human and simian sequences.

- *In Vivo* – Murine
- PIN-2 demonstrated antitumor activity in 3 metastatic, syngeneic, orthotopic mammary tumor models with varying degrees of immunogenicity indicating PIN-2 activity is a function of tumor antigenicity (4T1<TS/A<SM-1).

- *In Vitro* – Human Monocytes
- PIN-2 is a cell penetrating peptide (CPP) that rapidly enters and reprograms cellular transcription to promote maturation and function of monocyte derived dendritic cells is evidenced by:
  - Increases of surface co-stimulatory ligands CD80/CD86 (FACS)
  - Regulation of mRNA coding for immune response genes (Transcriptomics)
  - Increases of TNF-α cytokine secretion (ELISA)



Objectives

1[0] Translate preclinical observations predictive of PIN-2 immunomodulatory activity:
- Rapid on-set of activity (PK/PD)
- Early Immune activation signal in peripheral blood

2[0] To demonstrate safety and tolerability of bolus administration of PIN-2 to patients with advanced solid tumors who failed previous therapies.

Methods

Study Design

A first-in-human, open label, repeat dose study evaluating PIN-2 in subjects with solid tumors who had no potential curative options for their disease was conducted in 8 patients with extensively pretreated solid tumors to evaluate the pharmacodynamics (PD) and safety of PIN-2. The population selected for this study is adults with advanced solid tumors who had failed prior therapy and had a sufficient number of circulating lymphocytes and monocytes.

PIN-2 (300 µg) was administered intravenously, 3 times per week for two weeks followed by a one week rest period. Eligible subjects received a second cycle of treatment. During the first treatment cycle visits occurred everyday for 5 days, then every other day (to coincide with administration) for week 2, and at the end of week 3. In cycle two, visits occurred 3 times per week with administration, and at the end of week 6. Rich sampling was performed at the time of the first dose, with samples obtained during screening acting as the pre-dose sample, and at 0.25, 1, 2, 5, 10, 30 and 60 minutes post-dose.

Results

Baseline Characteristics

Primary Tumor Location (n=8)	PIN-2 Rx
Head/Neck	1
Prostate	1
Skin	1
Colorectal	3
Cholangiocarcinoma	1
Ovarian	1
Histologic Type	
Adenocarcinoma	5
Melanoma, malignant	1
Serous adenocarcinoma	1
Sqaumous cell carcinoma	1
Prior Treatment	
Surgery	6 (75%)
Radiation	5 (63%)
Systemic Therapy	8 (100%)
Prior lines of therapy (median [range])	4.5 (2,9)
Prior Immunotherapy	4

A total of 8 patients (2 male and 6 female), mean age 62.7 (±7.9) years, and a median of 4.5 prior treatment lines, were enrolled in 2 Australian centers. The study consisted of 2 cycles each comprised of 2 weeks of treatment 3 times per week with a 1 week rest period in between. The 2nd cycle of treatment was offered based on patient and investigator preference.

Pharmacokinetic Parameters

Parameter	Mean	SD	Median	Range
C_{max} (pg/mL)	77,500	61,600	53,300	17,500 – 176,000
T_{max} (min)	1.06	0.665	1.0	0.25 – 2.00
$AUC_{0\text{-}60}$ (pg-min/mL)	618,000	486,000	349,000	243,000 – 1,390,000
$AUC_{0\text{-}inf}$ (pg-min/mL)	690,000	493,000	464,000	276,000 – 1,480,000
$T_{1/2}$ (min)	24.0	8.07	23.5	14.2 – 40.2

PIN-2 was rapidly cleared from the vascular compartment which coincided with the appearance of an increase in circulating TNF-α at 6 hours post-administration. 2 patients developed anti-drug antibodies; however, they did not impact on the immuno-PD profile.

Results

*Biomarker Analysis**

Visit	TNF-α pg/mL	TNF- α CFB	CSF-2 pg/mL	CSF-2 CFB	IFN-γ pg/mL	IFN-γ CFB	IL-12 pg/mL	IL-12 CFB
Day 1 baseline	31.0 (15.6)		2.5 (2.6)		13.7 (27.1)		7.2 (7.6)	
Day 1 6h post-dose	71.2 (30.3)	40.2 (35.1)	2.4 (3.0)	0.2 (0.4)	3.9 (4.9)	-9.6 (25.4)	7.0 (7.8)	0.3 (1.6)
Day 22 6h post-dose	109.9 (88.5)	74.3 (93.1)	2.6 (3.3)	0.3 (0.5)	3.2 (4.3)	-14.7 (31.3)	9.9 (8.6)	0.9 (2.5)
Day 33 (end of study)	28.6 (19.0)		2.8 (3.4)		4.0 (4.6)		6.0 (5.9)	

Three circulating biomarkers were included in the primary endpoint: TNF-α, IFN-γ, and IL-12 with CSF-2 included as an exploratory biomarker. Pre- and 6 hour post-treatment values were obtained at the first dose of cycle 1 (day 1) and 6h post-dose on the first dose of cycle 2 (day 22). Table 2 displays values (mean ± SD) for selected visits: Day 1 baseline and 6 hours post-dose, day 22, 6 hours post-dose, and day 33 (end of study). Change from baseline (mean ± SD) is shown for the post-dose values. A strong signal of response to treatment in TNF-α at both infusions, with mean increases from baseline of 40.2 (35.1) and 74.3 (93.1) pg/mL was observed. The increase in TNF-α at Day 1 (6 hours post-treatment) is indicative of a primary stimulation whereas, Day 22 (6 hours post-treatment) suggests a "booster response". The remaining biomarkers demonstrated an inconsistent response at the sampling times evaluated. *Plasma was evaluated by validated enzyme-linked immunosorbent assay (ELISA).

Conclusions

Summary

1)This study showed a clear signal of treatment-associated response as indicated by marked increase in circulating TNF-α concentrations 6 hours after an infusion of PIN-2, which appeared to increase during a second cycle of treatment.

1)Mild to moderate infusion-related reactions (not uncommon) were observed and were readily managed with standard therapy.

1)The results were consistent with preclinical experiments evaluating the immunomodulatory properties of PIN-2 in the context of innate immune stimulation.

2) A primary stimulation and a "booster response" upon re-administration of PIN-2 following a 1 week rest period (Day 22) was observed.

3)PIN-2 is pharmacologically active, safe and well tolerated to support progression into additional clinical trials.

Translational/Clinical Significance

Dendritic cells are the master controllers that bridge innate and adaptive immunity and thus, play a central and pivotal role in promoting anticancer T-cell immunity. Immunostimulatory agents like PIN-2 that promote dendritic cell activation, maturation, and function should be utilized use in cancer immunotherapy strategies.

TNF-α is a pleiotropic cytokine proven to be (i) a key mediator of early innate immune signaling, (ii) indispensible as a regulator of antigen presentation to promote dendritic cell maturation and M1 macrophage polarization, (iii) as the component of a killing mechanism used by CD4+ helper T-cells and (iv) is critical for antitumor immunity and cancer immunotherapy.

The endogenous release of TNF-α is prerequisite for the induction of acute proinflammatory innate immune signaling. Systemic administration of TNF-α protein (exogenous) has limited clinical application due to toxicity, whereas, in contrast PIN-2 administration resulted in the endogenous release of TNF-α in the absence of obvious toxicity.

The *in vivo* pharmacokinetics of systemic PIN-2 administration observed in peripheral blood (elevated plasma TNF-α after 6 hours) demonstrate a rapid on-set of acute-phase innate immune activation can be considered an immuno-priming stimulus that can be restore the immune response against the tumor.

Induction of innate immunity by systemic administration of immunostimulatory agents represents a novel and unique approach to illicit antitumor immunity toward a wide array of tumor-associated antigens and neoantigens.

This study indicate that PIN-2 may have utility in combination therapy with existing treatment modalities (chemotherapy, radiation, I-O agents) to improve response rates in patients with advanced solid cancers.

Due to the short duration of this first-in-human trial, these results support conducting additional trials of longer duration.