

Clinical Results of the First-in-Man, Single-Ascending-Dose Study of PI-2301, a Second Generation Peptide Copolymer for the Treatment of Multiple Sclerosis

Joseph Kovalchin¹, Jeffrey Krieger¹, Ingrid Dufour¹, Kathy Collins¹, Michelle Genova¹, Michael Augustyniak¹, Kristen Rafuse¹, Tony Avri², Gwenola Gandon², Alain Patac³, Nicolas Fauchoux³, Uday Patel¹, Edward Mascioli¹, Eric Zanelli¹

¹Pepimmune, Inc. 64 Sidney Street, Suite 380 Cambridge, MA 02139, USA; ²Centre Eugène Marquis, rue de la Bataille Flandres-Dunkerque, 35042 Rennes, France; ³Biotrial S.A., rue Jean-Louis Bertrand, 35000 Rennes, France

Abstract

Objectives: PI-2301 is a novel compound in a class of autoimmune therapeutics called peptide copolymers. Copolymers are random mixtures of peptide sequences comprised of limited numbers of amino acids. Copaxone® is a copolymer which has been approved as a primary treatment for Relapsing Remitting-Multiple Sclerosis (RR-MS). PI-2301, like Copaxone, is an immunomodulator, which promiscuously binds to MHC Class II molecules and induces a skewed T_H2 T-cell response characterized by the activation and expansion of T-cells and monocytes which secrete IL-4, IL-5, IL-10, IL-13 and CCL22. This regulatory response is believed to interfere with the expansion of autoreactive T_H1/T_H17 cells. PI-2301 has shown superior therapeutic efficacy as compared to Copaxone in murine experimental allergic encephalomyelitis (EAE), an animal model that resembles multiple sclerosis, in both daily and weekly subcutaneous (SC) dosing regimens. The purpose of the present study was to evaluate the safety, tolerability, and early immunological effect(s) following SC administration of PI-2301 in a Single-Ascending-Dose, first-in-man study involving healthy, male adult volunteers.

Methods: The clinical study was designed in accordance with recommendations as defined in the Duff report and Committee for Human Medicinal Products (CHMP) guidelines issued in July 2007 for potentially immunomodulating therapeutics. Fifty-six subjects (eight cohorts of seven individuals; 5 active and 2 placebo) were given a single subcutaneous injection of PI-2301. The first dose was 0.01mg, which is 100-fold below the Minimal Anticipated Biological Effect Level (MABEL) and 50,000-fold below the No Observed Adverse Effect Level (NOAEL) in the most sensitive animal species tested with PI-2301. The parameters evaluated were safety, monitoring, pharmacokinetics, *in vitro* T-cell recall responses, antibody response to PI-2301, and changes in serum cytokines and chemokines.

Results: PI-2301 was generally well tolerated through the doses tested thus far (6 of the scheduled 8 doses). Evidence of immune priming (as shown by T-cell specific proliferative and cytokine responses) was observed at the projected MABEL dose in humans, i.e., 1mg. Further data collection and analyses are currently underway and will be available in the coming months.

Conclusions: This study represents the initial step in the development of an improved peptide copolymer with immunomodulatory properties for the treatment of multiple sclerosis.

Background

PI-2301 is a novel compound in a class of autoimmune therapeutics called peptide copolymers. Peptide copolymers are random mixtures of peptide sequences comprised of limited numbers of amino acids that are used as a primary treatment for autoimmune diseases such as multiple sclerosis. Peptide copolymers (Copaxone, PI-2301) interfere with the capacity of T-cells to recognize peptides presented by antigen-presenting cells (APCs) without creating a state of immunosuppression. Copolymers shift the immune response from a T_H1 to a T_H2 response.

PI-2301 is significantly more efficacious, when administered either on a daily or weekly regimen, in the treatment of EAE than Copaxone®. PI-2301 activates B-cells by up-regulating CD23 and CD86. PI-2301 directly acts on APCs to decrease TNF-α production and increase chemokines (CCL22 and CXCL13) production, which dampens the pro-inflammatory response and preferentially attracts T_H2 and T_{reg} cells. Increased serum concentrations of CCL22 and CXCL13 can be detected within minutes after SC administration of PI-2301 in a kinetics similar to the compound itself.

Objectives

To evaluate the safety, tolerability, and early immunological effect(s) following SC administration of PI-2301 in a Single-Ascending-Dose, first-in-man study involving healthy, male adult volunteers.

To evaluate the pharmacokinetic (PK) parameters (volume of distribution, serum clearance, mean residence time, ...) of PI-2301 in human serum using a newly validated proprietary method.

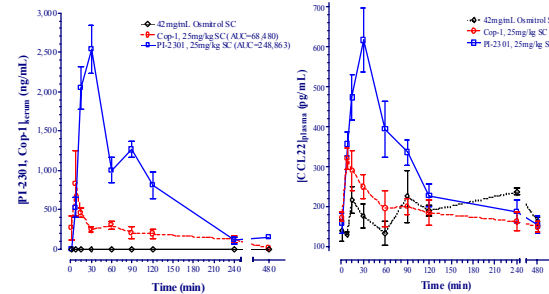
To use PK and PD parameters to establish a link between preclinical and clinical observations and to help us predict the most efficacious doses to be used in further clinical studies.

Study Design

- 8 cohorts of 7 subjects each; 56 total
- 5 active drug, 2 placebo in each cohort
- Dose range: 0.035 - 60 mg (0.035, 0.1, 0.3, 1, 3, 10, 30, 60 mg)
- 20 mg/mL solution, 1mL per injection site
- Healthy normal men
- Dosed sequentially (one subject per day, 3 weeks between start of each cohort)
- Safety review meeting after each cohort
- Review included clinical safety data, EKG, hematology, chemistry, urinalysis, trypsinase, immunologic markers

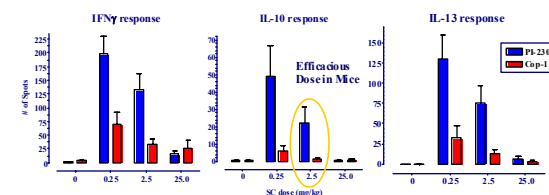
Preclinical Data

Serum Concentrations of PI-2301 vs Cop-1 and Plasma Concentrations of CCL22



Male CD-1 mice from CRL were dosed once subcutaneously with vehicle or 25mg/kg of PI-2301 or Cop-1. Blood was collected from individual mice at 1, 8, 15, 30, 60, 90, 120, 240, and 480 minutes after dosing. Serum and plasma were collected. Serum concentrations of PI-2301 and Cop-1 were measured using a proprietary pharmacokinetic assay. Plasma concentrations of CCL22 were tested using commercial ELISA assays.

PI-2301 and Copaxone Immune Priming in Mice



Female SIL mice from CRL were dosed subcutaneously once daily for five days with vehicle, 0.25, 2.5, or 25 mg/kg of PI-2301 or Cop-1. Splens were collected one week after the fifth dose. Splenocytes (4x10⁶) were restimulated with 1.2µg/mL of PI-2301 or Cop-1 for three days and ELISPOT was performed for IL-13, IL-10, and IFNγ.

Clinical Data

Phase Ia Trial – Predicted Serum Exposure

Total dose (mg)	Dose (mg/kg)	Increment factor	Predicted C _{max} (mg/mL)	Fold below C _{max} for NOAEL
NOAEL	25.000		3.180	
MABEL	0.0500		0.006	530
0.035	0.0005		0.0001	50,000
0.100	0.0014	2.86	0.0002	17,500
0.300	0.0043	3.00	0.0005	5,833
1.000	0.0143	3.33	0.0018	1,750
3.000	0.0429	3.00	0.0055	583
10.000	0.1429	3.33	0.0182	175
30.000	0.4286	3.00	0.0545	58
60.000	0.8571	2.00	0.1090	29

The table above shows the ascending doses in the SAD study design and their relation to No Observed Adverse Effect Level (NOAEL), The Maximum Recommended Starting Dose (MRSD) was 0.0005mg/kg or 0.035mg for a 70-kg human subject; this MRSD was 100-fold below the Minimum Anticipated Biological Effect Level (MABEL). For each dose, a predicted C_{max} was calculated using the formula (defined during our mouse PK studies):

$$C_{max} \text{ (ng/mL)} = 127.2 \times \text{SC dose (mg/kg)}$$

Each anticipated C_{max} was then compared to the observed C_{max} achieved in mice dosed with 25mg/kg (NOAEL).

Similarities Between Human and Monkey PK Parameters

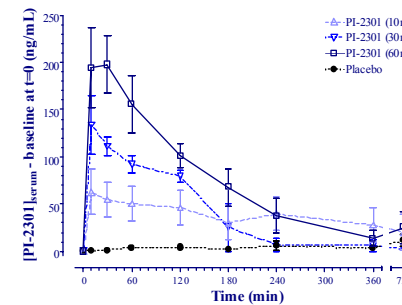
	CD-1 mice 40mg/kg SC	Cynos 40mg/kg SC	Humans 60mg (0.8mg/kg) SC
C _{max} (ng/mL)	3852	8275	217 (10850 for 40mg/kg)
T _{max} (min)	30	20	22
AUC _{last} (ng/mL·hr)	6782	16607	561 (28050 for 40mg/kg)
MRT _{last} (hr) mean residence time	1.8	4.1	2.7
F _{last} (%) bioavailability	15.8	14.8	-
T _{1/2el} (hr) elimination half-time	1.9	16.9	8.5
Cl _T /F (mL/hr/kg) serum clearance	5855	1831	1889
V _d /F (mL/kg) volume of distribution	15903	43480	5504

Treatment Emergent Adverse Events

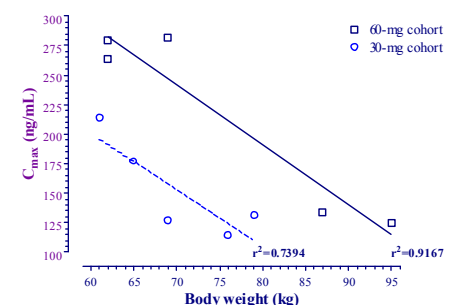
General conditions at sites of injections	Coh#4: 1.0 mg (N=5)		Coh#5: 3.0 mg (N=5)		Coh#6: 10.0 mg (N=5)		Coh#7: 30.0 mg (N=5)		Coh#8: 60.0 mg (N=5)							
	n	(%) AE	n	(%) AE	n	(%) AE	n	(%) AE	n	(%) AE						
All	2	40	2	3	60	9	5	100	15	5	100	22	5	100	31	3
Cyst	0	0	0	0	0	0	0	0	1	20	1	0	0	0	0	0
Erythema	0	0	0	3	60	3	5	100	7	5	100	10	5	100	15	
Induration	0	0	0	2	40	2	4	80	4	2	40	3	4	80	7	
Pain	0	0	0	1	20	1	0	0	0	0	0	0	3	60	6	
Pruritus	2	40	2	3	60	3	3	60	4	4	80	8	1	20	3	

Notes: Placebo (N=16) and active dose groups 0.35, 0.1, and 0.3 mg/subject (N=5 each) did not have any Adverse Events (AE). One individual experienced a rash in Cohort#5: 3.0 mg. In the same cohort a different individual experienced transient orthostatic hypotension.

Evidence of Serum Exposure Following PI-2301 SC Administration in Human Healthy Subjects

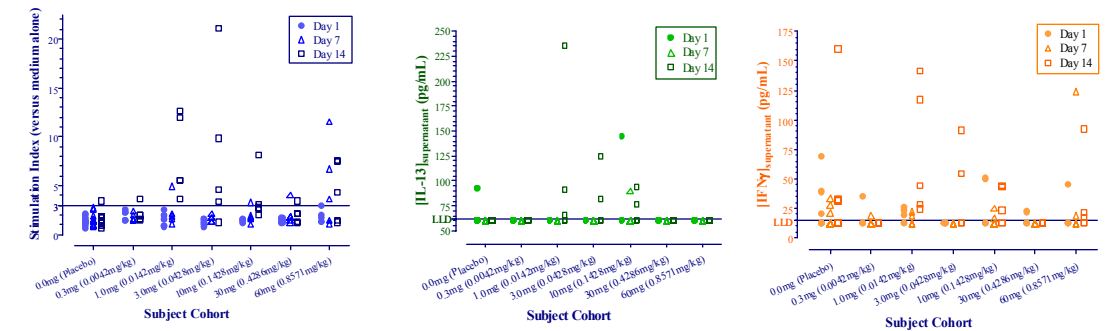


Correlation Between Body Weight and C_{max} in 30- and 60-mg Cohorts



Healthy male human subjects were dosed once subcutaneously with a single-ascending dose of PI-2301. Blood was collected from subjects at 1, 15, 30, 60, 120, 180, 240, 360, and 720 minutes after dosing. Serum concentrations of PI-2301 were measured using a proprietary PK assay. For subjects in Cohort#7 (30mg) and Cohort#8 (60 mg) a linear correlation was observed between PI-2301 serum concentrations and body weight.

Evidence of PI-2301 Immune Priming in SAD Subjects - PI-2301 Proliferation Assay, IL-13, and IFNγ Four-day Recall, 10µg/mL -



Blood was collected on day 1 (prior to compound administration) and on follow-up visits (day 7 and 14). Frozen PBMCs for all subjects in a given cohort and all time points were thawed at once and re-stimulated at 4x10⁵ PBMCs/well for 6 days with PI-2301, 10µg/mL. Supernatants were collected on day 6 prior to the addition of ³H-thymidine (proliferation assay) and tested for IL-13 and IFNγ production. Lower limit of detection: 62pg/mL for IL-13 and 15pg/mL for IFNγ.

Conclusions

- ✓ SC administration of PI-2301 in a Single-Ascending-Dose, first-in-man study involving healthy, male adult volunteers is safe and well tolerated.
- ✓ Mild and transient injection site adverse events were observed in a dose-dependent manner.
- ✓ We have developed pharmacokinetic assays that allow for the detection of PI-2301 and Cop-1 in serum.
- ✓ Serum levels of PI-2301 and Cop-1 correlate with plasma levels of CCL22.
- ✓ PI-2301 is more bioavailable than Cop-1 (could explain superior efficacy).
- ✓ Evidence of T-cell priming (antigen-specific proliferation and cytokine production) can be detected after a single administration PI-2301 in both animals and humans.
- ✓ No appreciable titers of PI-2301-reactive antibody were observed after a single compound administration in humans (data not shown).
- ✓ PI-2301 is better at generating a regulatory T-cell response (IL-10) when compared to Cop-1(could explain superior efficacy).
- ✓ Multiple-Ascending-Dose study is currently underway in Secondary Progressive MS patients.

