Preclinical and Clinical Development of a Second Generation Peptide Copolymer for the Treatment of Multiple Sclerosis

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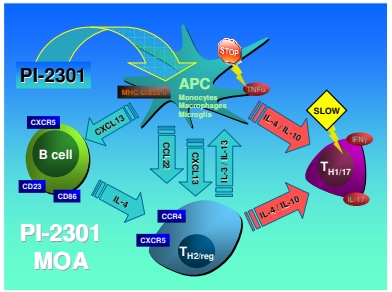
Abstract

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 that has been approved as a primary treatment for Relapsing Remitting-Multiple Sclerosis (RR-MS). PI-2301, like Copaxone, is an immunomodulator that binds MHC Class II molecules and induces a T_H2 response characterized by the activation and expansion of T-cells and monocytes producing IL-4, IL-5, IL-10, IL-13, and CCL22. This regulatory response is believed to interfere on the expansion of autoreactive T_H1/T_H17 cells. PI-2301 has shown superior efficacy as compared to Copaxone in experimental allergic encephalomyelitis (EAE), an animal model which resembles multiple sclerosis, in both daily and weekly dosing regimens. We now report the results of a Single-Ascending-Dose, first-in-man study evaluating the safety of PI-2301 in healthy, male adult volunteers. Fifty-six subjects (eight cohorts of seven individuals; 5 active and 2 placebo) were given a single subcutaneous injection of PI-2301. Design of this clinical study was in accordance with new recommendations as defined in the Duff report and recent CHMP guidelines issued in July 2007. The first dose was 100-fold below a well-defined Minimal Anticipated Biological Effect Level (MABEL) and 50,000-fold below the No Observed Adverse Effect Level (NOAEL) in the most sensitive animal species. Readouts included safety, pharmacokinetics, T-cell recall, and antibody response to PI-2301, serum cytokines and chemokines. This study represents the first 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.

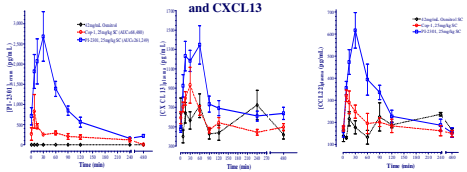
To use PK and PD parameters to establish a link between preclinical and clinical observations and to help us predict the most efficacious doses to be used in further clinical studies.

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, tryptase, immunologic markers

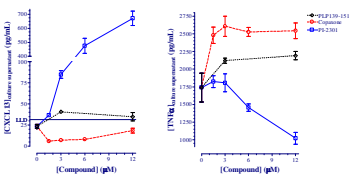
Preclinical Data

Serum Concentrations of PI-2301 vs. Cop-1 and Plasma Concentrations of CCL22 and CXCL13

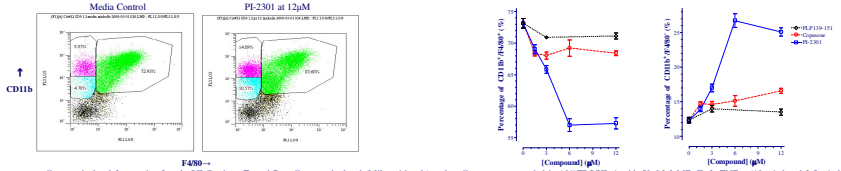


Male CD-1 mice from CRL were dosed once subcutaneously with Osmitrol or 25mg/kg of PI-2301 or Cop-1. Blood was collected from individual mice at 1, 8, 15, 30, 60, 90, 120, 240, and 480 minutes after dosing. Serum and plasma were collected. Serum concentrations of PI-2301 and Cop-1 were measured using a proprietary pharmacokinetic assay. Plasma concentrations of CCL22 and CXCL13 were tested using commercial ELISA assays.

Culture Supernatant Concentrations of TNF-α and CXCL13



FACS Analysis of Bone Marrow Derived Cells Cultured with PI-2301 or Cop-1 showing populations of $CD11b^+/F4/80^+$ and $CD11b^+/F4/80^-$



Bone marrow cells were isolated from naive female SJL/J mice. T- and B- cells were depleted (Miltenyi beads) and cells were resuspended in 10%FBS/HL-1 with 50μM 2-ME, IL-3, TNF-α (10ng/ml and 2.5ng/ml respectively; R&D Systems) with or without PI-2301, Copaxone®, or PLP139-151 peptide. Cultures were incubated at 37°C with 6%CO_2 in 24 well plates (1x10⁶ cells/well). After 6 days of culture, 1μg/ml LPS (Sigma) was added and the cultures were incubated for an additional two days. Culture supernatants were collected at SD7 and SD9 and tested for TNF-α and CXCL13 by ELISA (R&D Systems). Cells for FACS analysis were collected on SD1 both pre- and post- depletion. On SD9, cells were collected from the wells by scraping and where then stained for flow cytometric analysis using CD3:FITC, B220:PE, CD83:PE, CD11b:PerCP-CY5.5, and CD11c:PE-CY7 from BD; and F4/80:FITC from eBioscience.

Clinical Data

Phase Ia Trial – Predicted Serum Exposure

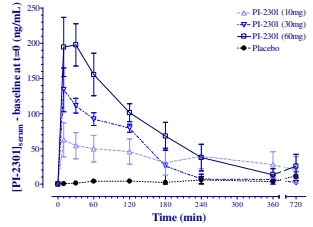
Total dose (mg)	Dose (mg/kg)	Increment factor	Predicted C_{max} (mg/mL)	Fold below C_{max} for NOAEL
NOAEL	25.000		3.180	
MABEL	0.0500		0.006	530
0.035	0.0005		0.0001	50,000
0.100	0.0014	2.86	0.0002	17,500
0.300	0.0043	3.00	0.0005	5,833
1.000	0.0143	3.33	0.0018	1,750
3.000	0.0429	3.00	0.0055	583
10.000	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 Cmax was calculated using the formula (defined during our mouse PK studies):

Cmax (ng/mL) = 127.2 x SC dose (mg/kg)

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

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



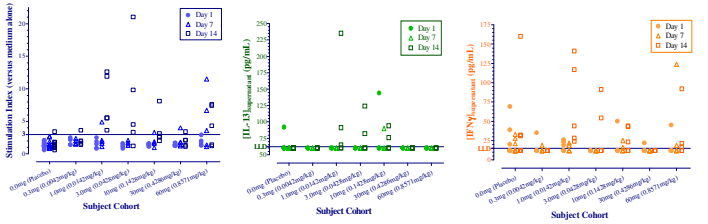
Healthy male human subjects were dosed once subcutaneously with a single-ascending dose of PI-2301. Blood was collected from subjects at 1, 15, 30, 60, 120, 180, 240, 360, and 720 minutes after dosing. Serum concentrations of PI-2301 were measured using a proprietary PK assay.

Treatment Emergent Adverse Events

General conditions at sites of injections	Cohl#4: 1.0 mg (N=5)			Cohl#5: 3.0 mg (N=5)			Cohl#6: 10.0 mg (N=5)			Cohl#7: 30.0 mg (N=5)			Cohl#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
Cyst	0	0	0	0	0	0	0	0	0	1	20	1	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 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 ^{3}H-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 and CXCL13.
- ✓ 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 of PI-2301 in both animals (data not shown) and humans.
- ✓ No appreciable titers of PI-2301-reactive antibody were observed after a single compound administration in humans. (data not shown)
- ✓ Multiple-Ascending-Dose study is currently underway in Secondary Progressive MS patients.