

Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose Trial of Synthetic Preimplantation Factor in Autoimmune Hepatitis

Christopher B. O'Brien,¹ Eytan R. Barnea,² Paul Martin,¹ Cynthia Levy,¹ Eden Sharabi,³ Kalyan R. Bhamidimarri,¹ Eric Martin,¹ Leopold Arosemena,¹ and Eugene R. Schiff¹

Preimplantation factor (PIF) is an evolutionary conserved peptide secreted by viable embryos which promotes maternal tolerance without immune suppression. Synthetic PIF (sPIF) replicates native peptide activity. The aim of this study was to conduct the first-in-human trial of the safety, tolerability, and pharmacokinetics of sPIF in patients with autoimmune hepatitis (AIH). We performed a randomized, double-blind, placebo-controlled, prospective phase I clinical trial. Patients were adults with documented AIH with compensated chronic liver disease. Diagnosis of AIH was confirmed by either a pretreatment International Criteria for the Diagnosis of AIH score of 15 or more, or a posttreatment score of 17 or more. Patients were divided into three dosing cohorts (0.1, 0.5, or 1.0 mg/kg) of 6 patients in each group. Three patients in each group had normal liver tests and 3 patients had abnormal liver tests. They were randomized to receive a single, subcutaneous dose of either sPIF or a matching placebo. Eighteen patients were enrolled, and all successfully completed the trial. There were no clinically significant adverse events and all doses were well tolerated. Ascending doses of sPIF produced a linear increase in the respective serum levels with a half-life of 90 minutes. There were no grade 2, 3 or 4 laboratory abnormalities. No patient developed detectable anti-sPIF antibodies. *Conclusion:* This first-in-human trial of the safety and pharmacokinetics of sPIF (a novel biologic immune modulatory agent) demonstrated both excellent safety and tolerability. The data support further studies of multiple ascending doses of sPIF in autoimmune hepatitis and potentially other autoimmune disorders. (*Hepatology Communications* 2018;2:1235-1246).

Autoimmune hepatitis (AIH) is a chronic liver disease of unclear etiology characterized by an inappropriate immune response directed against the liver. Manifestations include hypergammaglobulinemia, the presence of circulating autoantibodies, and necroinflammatory hepatic injury leading

to progressive fibrosis and cirrhosis.^(1,2) Despite the efficacy of present immune suppressive agents, many patients develop adverse reactions to their use limiting the proper dose or treatment duration. Ideal therapy for this condition would induce remission of disease while maintaining the patient's ability to protect

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIA, Clinical Laboratory Improvement Amendments; FDA, Food and Drug Administration; HIV, human immunodeficiency virus; IL, interleukin; NOAEL, no observed adverse effect level; PIF, preimplantation factor; PD-L1, programmed death-ligand 1; SAD, single ascending dose; sPIF, synthetic preimplantation factor; SQ, subcutaneously; and TNF, tumor necrosis factor.

Received January 8, 2018; accepted July 9, 2018.

Supported by BioIncept LLC and the Miami Center for AIDS Research (CFAR) at the University of Miami's Miller School of Medicine (NIH grant P30AI073961).

© 2018 The Authors. *Hepatology Communications* published by Wiley Periodicals, Inc., on behalf of the American Association for the Study of Liver Diseases. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial, and no modifications or adaptations are made.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep4.1239

Potential conflict of interest Dr. Bhamidimarri advises Gilead, Merck, AbbVie, Intercept, and Alexion. Dr. Martin consults and advises Bayer; he consults and received grants from Bristol-Myers Squibb and AbbVie; he received grants from Gilead and Merck. Dr. Barnea is employed, owns stock, and received grants from BioIncept LLC.

against opportunistic infections—without the presence of side effects from the treatment.⁽³⁾

Interestingly, pregnancy may provide important insights into the regulation of immunity and immune disorders.^(4–10) It has been noted that autoimmune disorders, including AIH, improve during pregnancy only to flare post-partum.^(5,11,12) Barnea et al. have identified a novel polypeptide preimplantation factor (PIF) that appears to be responsible for the mechanism of maternal immune tolerance to fetus during pregnancy.^(6,13) Although PIF's natural function appears to be the induction of maternal-fetal tolerance, its immunomodulatory properties suggest that it may have a very significant immunotherapeutic potential in a broad variety of diseases. PIF is a 15-amino acid polypeptide (MVRIKPGSANKPSDD) that is secreted by all viable (mammalian) embryos during intrauterine pregnancy.^(14,15) PIF acts to initiate and maintain intra-uterine (local) tolerance, but also has effects on the systemic maternal immune response—possibly explaining the remission of autoimmune diseases while pregnant.^(8,16–23) PIF is effective at low concentrations (nanomolar) in the blood with a universal cross-species (mammalian) effect.⁽²⁴⁾ We studied an exact analog of this native polypeptide, synthetic PIF (sPIF), which replicates native PIF action in both *in vitro* as well as *in vivo* preclinical studies.

Preclinical Studies

Initial preclinical studies demonstrate that PIF acts on both the innate as well as the adaptive arms of the human system and highlight a number of important immunological actions that are pertinent to its use as a potential therapy for autoimmune liver disease.

Immunological actions include both the control of inappropriate inflammation as well as the induction of immune tolerance through the modulation of cell-mediated immunity. Synthetic PIF modulates peripheral blood mononuclear cell activity by binding to CD14 (+) cells, shifting them from M1-inflammatory to M2-regulatory pattern.^(25,26) These macrophages act as antigen-presenting cells, which by upregulating B7-H1 (PD-L1) expression, downregulates activated T cells, blocking proliferation and reducing mixed lymphocyte reaction.^(8,9,13,27,28) By binding to the Kv1.3b channel of activated T and B cells, sPIF reduces K⁺ flux without affecting early Ca⁺⁺ flux, a hallmark of immune-suppressive agents.^(9,28) These Kv1.3b channels control both lymphocyte proliferation and the mixed lymphocyte reaction leading to a Th2/Th1 cytokine bias, while preserving the antipathogen Th1 cytokines.^(8,9,25) Synthetic PIF also regulates natural killer cells by downregulating CD69 expression, thus reducing their toxicity.⁽¹⁰⁾

Direct organ-specific effects are achieved by activation of protein disulfide isomerases that contain the antioxidant thioredoxin domain, protecting against oxidative stress. The activation of the heat shock proteins (HSP 70 and 90) and cochaperone BAG-3 prevents/corrects protein misfolding.⁽²⁷⁾ Interestingly, these target-organ-specific effects are modulated by binding a common RIP-K motif found in each protein pathway.^(9,27,29) This RIP-K is a serine-threonine kinase “death domain” protein that associates with the Fas antigen-blocking tumor necrosis factor (TNF) signaling cascades, preventing apoptosis and nuclear factor (NF)- κ B activation.

Synthetic PIF also has a multitude of effects on cytokine activity. Studies have demonstrated a significant reduction in the circulating levels of both

ARTICLE INFORMATION:

From the ¹University of Miami Schiff Center for Liver Diseases, Miami, Florida; ²BioIncept, New York, New York; ³Northwestern University Medical School, Chicago, Illinois.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Christopher B. O'Brien, M.D.
Divisions of Liver and GI Transplantation
University of Miami School of Medicine
1500 NW 12th Ave., Suite 1101
Miami, FL 33136

E-mail: cobrien@med.miami.edu
Tel: +1-305-243-6956
Fax: +1-305-243-6681

interleukin (IL)-1 α and IL-17. Levels of interferon (IFN)- γ needed for host protection are not affected.⁽²⁵⁾ Synthetic PIF also reduces inducible nitrous oxide synthetase expression in the liver. Finally, there is a decrease in the expression of CXCL2, which together with IL-1 β , reduces neutrophil chemotaxis, leading to decrease in both reactive oxygen species release and subsequent hepatocyte necrosis. The chemokine profiles of both CCL4 and CCL5, which are inducers of acute liver injury, are also diminished.^(25,30,31)

In summary, sPIF is a unique, first-in-class medication with a modulatory mechanism of action that is distinct from known immune suppressive agents such as cyclosporine, tacrolimus, sirolimus, or glucocorticosteroids. We report the first-in-human, randomized, double-blind, placebo-controlled, single-ascending dose study of sPIF in patients with AIH to assess safety, tolerability, and pharmacokinetics of this novel agent.

Patients and Methods

MATERIALS

The current Good Manufacturing Practice (cGMP)-quality sPIF, an exact analog of the human embryo-derived peptide (MVRKPGSANKPSDD), was synthesized at PolyPeptide Laboratories (San Diego, CA). Coldstream Laboratories (Lexington, KY) carried out the subsequent bottle and fill (15 mg sPIF/vial lyophilized) processing of the compound to drug substance. The sterile containers containing the lyophilized sPIF were stored at -20°C until use.

STUDY DESIGN

Our primary objectives were to examine the safety, tolerability, and pharmacokinetics of sPIF in patients with AIH. We designed the study as a phase I, randomized, double-blind, single-center, placebo-controlled trial to study the effect of single-ascending doses of sPIF. Because AIH involves fewer than 200,000 people in the United States, it could qualify for Orphan Drug designation. This special designation allowed the enrollment of patients with AIH in this trial, avoiding the need to expose healthy volunteers to the first-in-human use of an investigational agent. Our exploratory objectives were to evaluate the effect of sPIF on serum alanine aminotransaminase following administration of single, ascending, subcutaneously administered doses. In addition, serum (stored at -80°C $\pm$ 10°)

was collected for possible pharmacodynamic studies, including possible genomic, proteinomic, and cytokine analyses in the future.

Dosing was completed in three sequential cohorts of 6 patients (3 patients with *normal liver biochemical tests* and 3 patients with *abnormal liver biochemical tests*). The presence of abnormal liver biochemical test(s) was defined by an elevation in the serum alanine aminotransaminase (ALT) level above the upper limits of normal of the reference range of the laboratory.

Normal Liver Biochemical Tests

Within each dosing cohort (3 patients/cohort; 9 subjects in total), patients with normal liver biochemical tests were randomized in a 2:1 ratio (active drug: placebo) to receive a single dose of sPIF or placebo:

- Cohort 1: single dose 0.1 mg/kg sPIF or placebo day 1 given subcutaneously (SQ)
- Cohort 2: single dose 0.5 mg/kg sPIF or placebo day 1 given SQ
- Cohort 3: single dose 1.0 mg/kg sPIF or placebo day 1 given SQ

Abnormal Liver Biochemical Tests

Within each dosing cohort (3 patients/cohort; 9 subjects in total), patients with abnormal liver biochemical tests were randomized in a 2:1 ratio (active drug : placebo) to receive a single dose of sPIF or placebo:

- Cohort 1: single dose 0.1 mg/kg sPIF or placebo day 1 given SQ
- Cohort 2: single dose 0.5 mg/kg sPIF or placebo day 1 given SQ
- Cohort 3: single dose 1.0 mg/kg sPIF or placebo day 1 given SQ

PATIENTS

Inclusion Criteria

We included patients from ages 18 to 75 years old, of non-child-bearing potential (to avoid the possibility of antibodies being formed to sPIF, in which case future fertility might be impaired in patients of child-bearing potential). Subjects had AIH without hepatic decompensation. The diagnosis of AIH was confirmed at screening by either a pretreatment

TABLE 1. CONCOMITANT IMMUNOSUPPRESSION MEDICATION AND DOSE

Pt #*	Budesonide	Prednisone	Azathioprine	Ursodiol	Mycophenolate	LFTs	Received	Group†
001					1000	Abnl	Plac	0.5
002			50	1000		Norm	0.1	0.1
003				1000		Abnl	Plac	0.1
004				500	2000	Norm	Plac	0.1
005				1000		Abnl	0.1	0.1
007				1000		Norm	0.1	0.1
008				1000		Abnl	0.1	0.1
009				500		Norm	Plac	1
010			100			Norm	Plac	0.5
011		2.5	100			Norm	0.5	0.5
012						Abnl	0.5	0.5
013		20	100			Abnl	0.5	0.5
014	3					Norm	0.5	0.5
016				900		Norm	1	1
018				1000	1000	Abnl	1	1
019		10			2000	Abnl	1	1
022			50			Norm	1	1
023		5	100			Abnl	Plac	1

Note: Medications are in milligrams.

Abbreviations: Abnl, abnormal; LFTs, liver biochemical/function tests; Norm, normal.

*The assigned screening number.

†The assigned dosing group (0.1, 0.5, or 1.0 mg/kg sPIF).

score of 15 or more or a posttreatment score of 17 or more by the International Criteria for the Diagnosis of Autoimmune Hepatitis.⁽³²⁾ All patients who were enrolled in the study had received standard initial therapy with prednisone and azathioprine (or mycophenolate if intolerant to azathioprine) after their initial diagnosis of AIH and had normalized their ALTs. Treatment with oral, immunosuppressive drug(s) had

to be unchanged for at least 6 weeks prior to screening in all patients, whether in the normal or abnormal ALT cohorts. Permitted concomitant medications included ≤ 100 mg azathioprine per day, ≤ 9 mg budesonide per day, $\leq 3,000$ mg mycophenolate mofetil per day, ≤ 30 mg prednisone per day, ≤ 1500 mg ursodeoxycholic acid per day, and ≤ 6 mg tacrolimus per day. (See Table 1 for the actual immunosuppression by patient.)

Exclusion Criteria

Decompensated liver disease was defined on the basis of any one of the following laboratory values at the screening evaluation: total bilirubin $> 1.5 \times$ upper limits of normal (ULN), prothrombin time $> 1.2 \times$ ULN, platelets $\leq 100,000/\text{mm}^3$, or albumin $< 3 \text{ g/dL}$ or history of clinical hepatic decompensation (e.g., ascites, jaundice, encephalopathy, or variceal hemorrhage). Patients also could not have a hemoglobin of less than 11 g/dL at the screening evaluation, serological evidence of HIV infection, or evidence of hepatocellular carcinoma (i.e., screening α -fetoprotein $> 50 \text{ ng/mL}$ or other standard of care measure). Subjects with, or a history of, clinically significant oncologic, pulmonary, hepatic, gastrointestinal, renal, other cardiovascular, metabolic, endocrine, neurologic, immunologic, or hematologic illness or any other major medical disorder were excluded from participation in this trial. Patients could not have received treatment with potentially hepatotoxic drugs within 3 months (90 days) prior to day 1, could not receive chemotherapeutic agents, or could not have had a change in their immunosuppressant dosing during the study for any medical condition.

ASSESSMENTS

Safety and Tolerability

Safety and tolerability were evaluated by assessment of clinical laboratory tests, periodic physical examination, including vital signs measurements, and 12-lead electrocardiogram at baseline (predose, day 1) and at various time points during the treatment phases of the protocol, and by the documentation of adverse events. Concomitant medication intake was also recorded. All adverse events and all treatment-related adverse events were listed by subject. Adverse events were summarized by relationship to study drug and severity.

Laboratory Studies

The standard comprehensive metabolic profiles, complete blood counts, and the standard laboratory measurements were carried out at the University of Miami Transplant Laboratory. During the initial screening period, patients were categorized as normal or abnormal by their ALT levels. Patients' serum samples were collected and analyzed at five different time points: screening (day -28 to day 0), pre-injection (day 0), and postinjection days 1, 2, and 8.

Pharmacokinetics

The pharmacokinetic analysis was performed by determining sPIF levels in the plasma collected at baseline, 30 (± 5), 60 (± 10), 120 (± 15), and 240 (± 20) minutes after SQ injection using validated liquid chromatography and mass spectroscopy assay (lower limit of quantification 1 ng/mL). Because sPIF is administered subcutaneously, absorption into the systemic vascular system must take place. Postdistribution serum concentrations were used to calculate the pharmacokinetic constants, volume of distribution, elimination rate constant, and half-life of the drug. The elimination rate constant (k_e) was computed using the following equation: $k_e = -(\ln C_1 - \ln C_2)/(t_1 - t_2)$.⁽³³⁾ The elimination rate constant was converted into the half-life using $t_{1/2} = 0.693/k_e$. We used the quotient of the dose and the extrapolated plasma concentration at time 60 minutes to calculate the hybrid constant volume of distribution/bioavailability. The extrapolated plasma concentration at time zero was calculated using a variant of the intravenous bolus equation: $C = C/e^{-k_e t}$, where t and C are the times/concentration pair that occur after administration of the extravascular dose in both the postabsorption and postdistribution phases. Dose proportionality information was obtained by comparing plasma levels of sPIF across all dose levels evaluated, across applicable cohorts.

Anti-sPIF Antibody

The ELISA to detect the immunogenicity of sPIF in human was developed and validated at a College of American Pathologists (CAP) accredited, Clinical Laboratory Improvement Amendments (CLIA) certified laboratory: the Genway Biotech (San Diego, CA). The testing of the clinical trial specimen was done at the Hepatology Research Laboratory, University of Miami (Miami, FL), a CLIA-certified laboratory by a CLIA-approved and CAP-approved personnel (Sivakumar Ramu, Ph.D.). The assay was developed by the adding the purified rabbit anti-sPIF IgG to pooled human male serum. The assay uses Affibody, a synthetic protein A, which binds to both the Fc and Fab portions of rabbit, human, and mouse IgG. When rabbit anti-sPIF antibody is added or if human anti-sPIF antibody is present they will bind to sPIF coated in a plate. The biotin-labeled Affibody binds to captured rabbit or human IgG. This complex is then detected using horseradish peroxidase-labeled avidin. The amount of antibody bound is proportional to the intensity of the absorbance and is measured using the spiked rabbit anti-sPIF antibody as standard.

Exploratory Assays

Immune cells and plasma (stored at $-80^{\circ}\text{C} \pm 10^{\circ}\text{C}$) were also collected for pharmacodynamic studies including cytokine analyses and potential future genomic and proteomic studies. Cytokines IL-1 β , IL-4, IL-8, IL-10, IL-17 α , IL-17 ϕ , IL-21, IL-22, IL-23, IFN- γ , intraperitoneal-10, monocyte chemoattractant protein-1, and TNF- α were determined in undiluted plasma using MILLIPLEX multiplex cytokine magnetic bead panels (EMD Millipore, Burlington, MA) in the MAGPIX instrument (Luminex, Austin, TX). Median fluorescent intensities were analyzed with MILLIPLEXTM Analyst Software (EMD Millipore) and cytokine levels were expressed as picograms per milliliter.

Interventions

The randomization schedule showing the assignment of active drug versus placebo within each part/cohort was provided by the statistician to the unblinded pharmacist at the University of Miami, who was responsible for dispensing the study drug to individual subjects. The assignment of the randomization placebo versus active drug was made by the pharmacy. Synthetic PIF was reconstituted in the pharmacy from powder to a 0.5 mL total volume per syringe with Lactated Ringer's Injection, USP (0.1, 0.5, 1 mg/kg), with the dose appropriate for the patient's weight. Lactated Ringer's Injection, USP 0.5 mL, was used as the placebo and was administered subcutaneously to subjects randomized to receive placebo under the same conditions described previously for active drug recipients.

Approval and Consent

The University of Miami Institutional Review Board performed a priori approval of the study. All patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. An internal (University of Miami) data and safety monitoring board reviewed the safety and tolerability before advancing to the next dosing level.

STATISTICAL ANALYSIS

Safety and pretreatment (i.e., screening and baseline [predose, day 1]) data were analyzed by the frequency of events/abnormalities or descriptive statistical

summaries (e.g., number of subjects [n], mean, SD, median, and range). These data were compiled into listings by subject, and tabular summaries were prepared. The authors performed the data analysis.

Results

Patients were enrolled at one site in the United States from September 2014 to April 2016. We reviewed 252 patients' charts: 23 agreed to be screened, 1 declined to participate after receiving a randomization number, and 18 were enrolled in the study. Twelve were randomly assigned to the active drug sPIF, and 6 to the placebo treatment arm. All patients who received 1 dose of drug or placebo completed the trial (Fig. 1).

BASELINE CHARACTERISTICS

The score for AIH was greater than 17 in all patients following treatment and greater than 15 in patients before treatment, according to the Revised International Diagnostic Criteria for Autoimmune Hepatitis.⁽³²⁾ The sPIF and the placebo groups were similar in terms of demographic and baseline characteristics. All participating patients were female, and the majority were Caucasian. The average age was 60 years old in the placebo and the three dosing groups of sPIF. Baseline liver biochemical tests were similar between the groups receiving the active drug versus the comparative placebo group. More details regarding the baseline characteristics can be found in Table 2.

SAFETY AND TOLERABILITY

There were no grade 2, 3, or 4 adverse events in any of the 12 body systems reported by symptoms or in the physical exam in any patient to date during the active dosing portion of the study or in the 7-day follow-up period. One patient had headache that spontaneously resolved. One episode of "liver fullness" sensation transiently occurred in 1 patient. Otherwise, no clinical side effects by history or physical exam were present in any dosing group.

There have been no grade 2, 3, or 4 adverse events of laboratory values in any patient to date during the single dose portion of the study or in the 7-day follow-up period. Minor, grade 1, or nongraded (World Health Organization criteria) changes in the blood

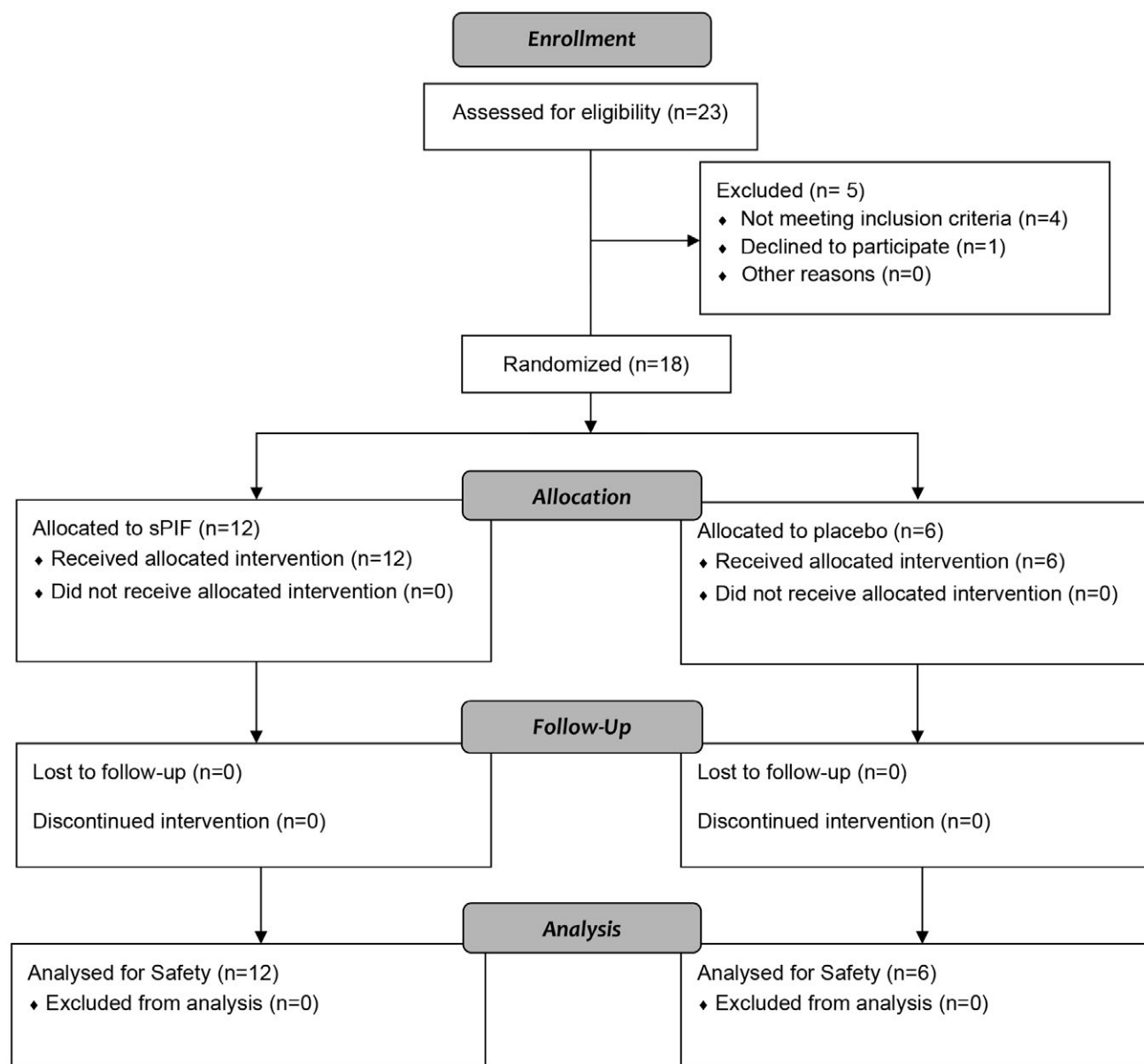


FIG. 1. Schematic representation of double-blind, randomized, controlled, single-center, phase I study that evaluated the SADs of sPIF against placebo in AIH (CONSORT flow diagram). Of the 23 patients screened, 18 were eligible for the study protocol. Patients were divided into one of three dosing groups: 0.1, 0.5, and 1.0 mg/kg of sPIF. Patients (n = 6) within each dose were randomized to receive that dose of sPIF (n = 4) or a matching placebo (n = 2). All patients received their designated active drug or a matching placebo. No patients were lost to follow-up or discontinued intervention. All patients were randomized and analyzed for safety, and none were excluded from the analysis.

studies occurred in the following patients (listed by screening number) from baseline values. The overall adverse events and their frequency by patient number are listed in Table 3. There were no clinically significant laboratory results that required the study drug to be modified, interrupted, or discontinued.

PHARMACOKINETICS

Synthetic PIF demonstrated dose-proportional changes in the level of drug in the range of doses used in this study (0.1, 0.5, and 1.0 mg/kg). The increases in $C_{\max}$ values were generally greater than dose, proportionally.

TABLE 2. DEMOGRAPHICS AND BASELINE CHARACTERISTICS

	Placebo	sPIF	sPIF	sPIF	sPIF
	All	All	0.1 mg/kg	0.5 mg/kg	1.0 mg/kg
Female, n (%)	6 (100%)	12 (100%)	4 (100%)	4 (100%)	4 (100%)
Age (y), mean $\pm$ SD (mean)	60 $\pm$ 11	60 $\pm$ 9	63 $\pm$ 10	59 $\pm$ 8	59 $\pm$ 9
Ethnicity					
Caucasian, n	3 (50%)	11 (91%)	4 (100%)	3 (75%)	4 (100%)
Hispanic, n	1 (17%)	1 (9%)	0 (0%)	1 (25%)	0 (0%)
Black, n	2 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Liver function tests					
TB (mg/dL), mean $\pm$ SD	0.6 $\pm$ 0.3	0.7 $\pm$ 0.2	0.6 $\pm$ 0.2	0.7 $\pm$ 2	0.7 $\pm$ 0.2
AP (IU/L), mean $\pm$ SD	106 $\pm$ 14	89 $\pm$ 18	91 $\pm$ 17	82 $\pm$ 19	96 $\pm$ 21
ALT (IU/L), mean $\pm$ SD	66 $\pm$ 18	59 $\pm$ 33	57 $\pm$ 31	77 $\pm$ 46	42 $\pm$ 9
AST (IU/L), mean $\pm$ SD	58 $\pm$ 26	52 $\pm$ 26	46 $\pm$ 23	62 $\pm$ 35	47 $\pm$ 21
Albumin (g/dL), mean $\pm$ SD	4.3 $\pm$ 0.3	4.3 $\pm$ 0.2	4.3 $\pm$ 0.2	4.3 $\pm$ 0.1	4.2 $\pm$ 0.3
γ globulin (U/L), mean $\pm$ SD	3.8 $\pm$ 1.5	3.5 $\pm$ 0.3	3.4 $\pm$ 0.1	3.6 $\pm$ 0.3	3.5 $\pm$ 0.4
Serologies					
Anti-HAV (positive), n					
Anti-HBsAg (positive), n	0	0	0	0	0
Anti-HCV (positive), n	0	0	0	0	0
AIH score, mean $\pm$ SD (mean)	18.2 $\pm$ 1.5	18.8 $\pm$ 1.2	18.8 $\pm$ 1.5	18.5 $\pm$ 1.0	19.0 $\pm$ 1.4

Abbreviations: AP, alkaline phosphatase; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; TB, total bilirubin; γ globulin, gamma globulin.

The maximum blood level was 9.4 ng/mL. The lowest, 0.1-mg/kg sPIF dose, did not produce a detectable blood level by the present assay (Table 4). Using these parameters, we found that the $t_{1/2}$ of sPIF SQ was 91 minutes. In a similar fashion, the volume of distribution was 22 L using a 1-compartment model assumption. Figure 2 demonstrates the time course of the plasma level of sPIF in the 5 patients whose blood was available for analysis. Levels peaked between 30 minutes and 60 minutes SQ. The level of sPIF was essentially nondetectable in the plasma by 4 hours after injection.

ANTI-SPIF ANTIBODY

We tested 18 multiple ascending dose samples (the second trial to be submitted later for publication), beginning day 1, day 15, and day 29, and single ascending dose (SAD) patients. Each sample was tested in triplicate and the assay was repeated 3 times to confirm the results. None of the samples tested had a detectable level of IgG against sPIF.

EXPLORATORY ANALYSES

There was a planned exploratory analysis for a potential assessment of efficacy in place at the start of

the trial. Components of this analysis included evaluation of the effect of sPIF on serum ALT following administration of single ascending, subcutaneously administered doses. Figure 3 shows ALT and AST levels in patients with normal liver function comparing baseline to 24 hours post injection.

CYTOKINE ANALYSIS

Paired serum samples were available from all 18 patients to study the effect of sPIF on serum chemokines and cytokines. There was a trend toward increased concentrations of the 13 chemokines and cytokines examined with increasing doses of sPIF. However, none of them were of such a magnitude to demonstrate any significant directional pattern of change (Table 5).

Discussion

We report the first-in-human trial exploring the safety and pharmacokinetics of sPIF, a novel biologic immune modulatory agent. The major elements for determining the maximum recommended starting dose of sPIF included determining the no observed adverse effect level (NOAEL) and conversion of the

TABLE 3. LISTING OF TREATMENT-EMERGENT LABORATORY ABNORMALITIES

Patient No.	Abnormality	Normal Range	Grade
001	Decrease HGB 10.3	(Normal 11.2-15.7)	Grade I
002			
003			
004			
005	Increase PTT 42.1	(Normal 25.1-36.5)	Grade I
006			
007			
008			
009			
010	Urine 7 rbc/hpf	(Normal < 3 rbc/hpf)	Grade I
011	Decrease WBC 2.5	(Normal 3.98-10.04)	Grade I
	Decrease HGB 10.6	(Normal 11.2-15.7)	Grade I
012			
013	Increase ALT 84	(Normal 13-69)	Grade I
014	Increase AST 47	(Normal 15-46)	Grade I
016	Increase amylase 138	(Normal 30-110)	Grade I
017			
018			

Abbreviations: HGB, hemoglobin; PTT, partial thromboplastin time; rbc/hpf, red blood cells/high power field; WBC, white blood cells.

NOAEL to the human equivalent dose (HED) after the application of a safety factor (usually at least 10). In independent toxicology studies in both mice and dogs, the NOAEL was found with sPIF doses at 400 mg/kg

TABLE 4. MEAN PHARMACOKINETICS VALUES AFTER A SINGLE SUBCUTANEOUS DOSE OF SPIF

	sPIF	sPIF	sPIF
	0.1 mg/kg	0.5 mg/kg	1.0 mg/kg
PK parameter	(n = 4)	(n = 4)	(n = 4)
Mean C_{max} , ng/mL	NA*	3.7	9.4
Mean T_{max} , min	NA	30	30
Mean $T_{1/2}$, min	NA	63	109
Mean C_{last} , ng/mL	NA	0	0.5
Mean T_{last} , min	NA	240	240

*"NA" indicates that there were insufficient data for the 0.1-mg/kg dosing cohort because of the inability to detect serum values of sPIF at this dose.

Abbreviations: C_{max} , maximum concentration; PK, pharmacokinetics; T_{max} , half-life time taken to reach maximum concentration; $T_{1/2}$, half-life; C_{last} , last observed quantifiable serum concentration of the drug; T_{last} , time (observed time point) of C_{last} .

for mice and 40 mg/kg in dogs for 2 weeks followed by 2 weeks follow-up. The common conversion factors for deriving a HED yielded a starting dose of 0.1 mg/kg of sPIF in the SAD portion of the protocol. However, an alternative approach is also available that places primary emphasis on preclinical pharmacokinetics and modeling. In both the autoimmune and transplant models, doses of 0.1-1 mg/kg had the maximum efficacy.^(25,26,34,35) This confirmed our use of the 0.1-mg/kg starting dose for the SAD study.

We found that in this SAD study in patients with AIH, doses of sPIF ranging from 0.1, 0.5, and 1 mg/kg administered subcutaneously demonstrated both

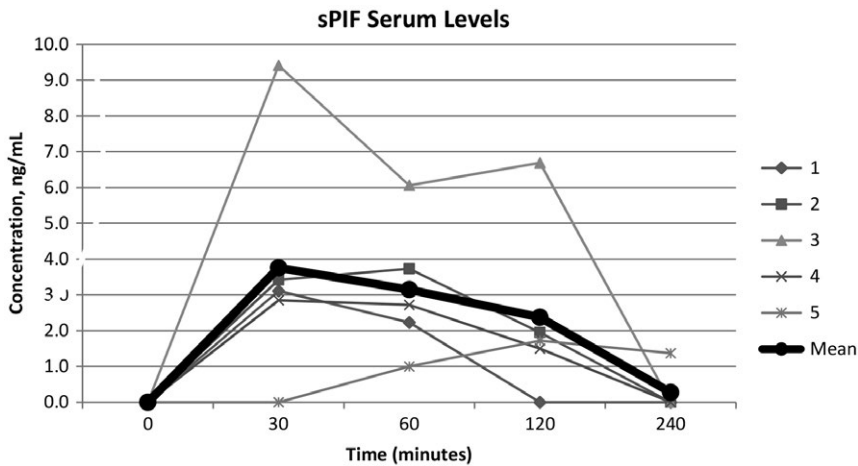


FIG. 2. Time-courses of individual patients (values available for analysis) and mean sPIF serum levels following a single subcutaneous dose of sPIF.

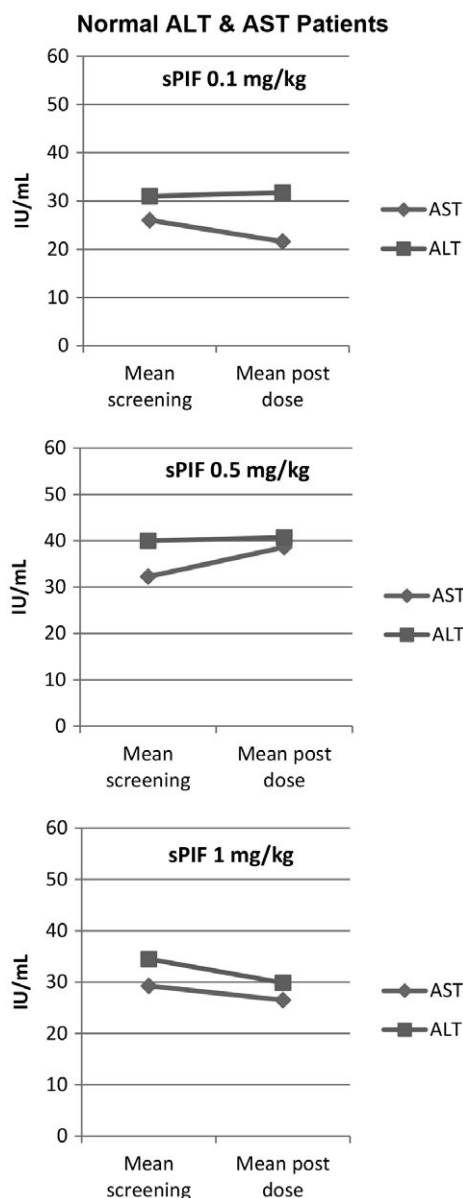


FIG. 3. Mean values of serum ALT and AST levels at baseline (mean screening) and 24 hours following (mean post dose) a single dose of sPIF given subcutaneously.

excellent safety and tolerability. There were no grades 2, 3, or 4 adverse events noted in any of the dosing cohorts. The only two reported study-related adverse events were headache ($n = 1$) and a sensation of abdominal fullness ($n = 1$) that spontaneously resolved within 1 hour after sPIF administration. Most importantly, there were no dose-dependent trends in adverse events. As expected, because sPIF is a synthetic analog of a naturally occurring biological peptide present during human pregnancy, we found sPIF to be extremely

TABLE 5. CYTOKINE AND CHEMOKINE CHANGES FOLLOWING SPIF

sPIF	Pretreatment	0.1	0.5	1
IFN- γ	158	16%	-17%	52%
1L-10	956	50%	50%	50%
IL-17 α	47	32%	6%	53%
IL-1 β	3822	50%	50%	50%
IL-4	542	49%	49%	51%
IL-8	5320	48%	49%	62%
IP-10	3043	41%	43%	58%
MCP-1	13,414	45%	45%	60%
TNF- α	1264	49%	49%	52%
IL-21	229	23%	22%	62%
IL-17 ϕ	0	0%	0%	0%
IL-22	4	25%	25%	50%
IL-23	101	28%	22%	56%

Note: Serum cytokine and chemokine induction following the dosing in patients assigned to the four different dosing cohorts of sPIF: 0, 0.1, 0.5, and 1.0 mg/kg, respectively.

Abbreviations: IP, inducible protein; MCP, monocyte chemotactic protein.

well-tolerated. Our ELISA assay also did not find any detectable level of IgG against sPIF (anti-sPIF antibody).

Synthetic PIF had a dose-proportional change in plasma C_{max} concentration of drug in the range of doses used in this study (0.1, 0.5, and 1.0 mg/kg). The maximum blood level in our patients on the 1-mg/kg dose was 9.4 ng/mL. The sPIF dose of 0.1 mg/kg did not produce a detectable blood level by the present assay. The median plasma half-life across the dosing cohorts was 91 minutes. Levels peaked between 30 minutes and 60 minutes after the subcutaneous injection. The level of sPIF was essentially nondetectable in the plasma by 4 hours after injection. However, the pharmacokinetics of the plasma half-life are shorter in duration than the apparent pharmacodynamic effects of this novel agent *in vivo* based on the preclinical study results.

Both preclinical *in vitro* as well as *in vivo* studies of sPIF have demonstrated that it exerts its immunologic activity through a variety of innate and adaptive immune actions. The immunological actions include both control of inappropriate immune activation and induction of immune tolerance. Synthetic PIF controls CD14 (+) macrophages, shifting them from a M1-inflammatory to M2-regulatory pattern.^(25,26) Synthetic PIF has been shown by flow cytometry to modulate activated T cells and B cells by binding to the Kv1.3b channel. This Kv1.3b channel binding is characteristic of

immunosuppressive drugs and reduces K^+ flux without changing early Ca^{++} flux. Beyond targeting and protecting against oxidative stress, sPIF also regulates Kv1.3b channels, to reduce K^+ flux as cortisone does, acting as a competitive inhibitor.^(9,28) Evidence for immune regulation without overt immunosuppression is demonstrated by a decrease in the mixed lymphocyte reaction that is consistent with a Th2/Th1 cytokine bias, while simultaneously preserving antipathogen Th1 cytokines. Synthetic PIF downregulates natural killer cells by decreasing CD69 expression, diminishing their activity.⁽¹⁰⁾ We did not have serum IgG levels to analyze per protocol; however, this would be important to monitor in the future as a marker of B-cell activity.

This immune modulation is associated with a compensatory change in cytokine and chemokine release.^(8-10,27) This was apparent in a graft versus host disease (GvHD) murine model in which sPIF reduces circulating IL1 β and IL17, without affecting interferon- γ levels, resulting in the preservation of antipathogen actions.⁽²⁵⁾ Synthetic PIF decreases in the expression of CXCL2, which together with IL-1 β , prevents neutrophil chemotaxis, decreasing reactive oxygen species release and the subsequent hepatocyte necrosis. Both CCL4 and CCL5 (which are inducers of acute liver injury) are also diminished.⁽²⁵⁾ Overall, the *in vitro* time-course of these immune system effects show that, despite rapid clearance sPIF from the plasma, the pharmacodynamic effects persist long after the sPIF is no longer detectable. Interestingly, we found a moderate increase in both profiles of pro-inflammatory as well as the anti-inflammatory cytokines in our patient populations (Table 5). It may be possible that higher doses of sPIF would result in a decrease in the TNF and IL-23 levels.

Other activities of sPIF include the binding through core Receptor-interacting serine/threonine-protein kinase sequence to protein di-sulfide isomerase which contains the antioxidant protein thioredoxin to decrease oxidative stress and heat shock proteins (HSP 70, 90) and the co-chaperone BAG-3 cascade, preventing protein misfolding.⁽²⁷⁾

In vivo studies of this novel immune modulatory polypeptide have revealed beneficial effects in diverse preclinical immune disorder models of neuroinflammation, vascular inflammation, juvenile diabetes, and ionic radiation.^(26,28,34-37) Short-term, low-dose sPIF administration in a GvHD murine model prevented skin and colon ulceration, and most relevant to this study of AIH, sPIF protected against active liver inflammation (documented by both histology and

local cytokine and chemokine expression). This persisted for several months after stopping therapy.^(25,30,31) However, the hypothesis that this model would predict that sPIF and be used in the treatment of patients with AIH had not been studied to date. To assess a possible marker of efficacy, we had a planned exploratory proof of concept (activity) in place at the start of the trial. Our major focus was to evaluate a possible proof of concept that sPIF would improve the serum ALTs in the patient cohorts that had abnormal liver biochemical tests at baseline. We found no significant decrease in the either of the serum transaminases (ALT and AST) the days after administration of the sPIF dose when compared with the patients who received the placebo. However, this was only after a single dose, and the maximum blood level of sPIF in our patient (9.7 ng/kg) was significantly less than that found in a healthy human female during pregnancy. This result suggests that multiple doses or higher doses than 1 mg/kg sPIF may need to be used in future phase II efficacy studies. In conclusion, our data demonstrate that sPIF is safe, well-tolerated, and met the primary endpoints of the study. The data support a planned study of multiple ascending doses of sPIF in AIH.

Acknowledgment: We thank Odalys Rodriguez Bravo, RN, and Eva Pavicic, RN.

Author contributions: The study was designed and conducted by the lead author in collaboration with co-investigators. The lead author designed the study, collected the data, monitored study conduct, and performed statistical analyses. All authors had access to the study data and have reviewed and approved the final manuscript.

REFERENCES

- 1) Manns MP, Vogel A. Autoimmune hepatitis, from mechanisms to therapy. *Hepatology* 2006;43:S132-S144.
- 2) Van Gerven N, de Boer Y, Mulder C, van Nieuwkerk C, Bouma G. Auto Immune hepatitis. *World J Gastroenterol* 2016;22:4651-4661.
- 3) Czaja AJ, Freese DK. American Association for the Study of Liver Diseases. Diagnosis and treatment of autoimmune hepatitis. *Hepatology* 2002;36:479-497.
- 4) Barnea ER, Rambaldi M, Paidas MJ, Mecacci F. Reproduction and autoimmune disease: important translational implications from embryo-maternal interaction. *Immunotherapy* 2013;5:769-780.
- 5) Paidas MJ, Annunziato J, Romano M, Weiss L, Or R, Barnea ER. Pregnancy and multiple sclerosis (MS): a beneficial association. Possible therapeutic application of embryo-specific Pre-implantation Factor (PIF*). *Am J Reprod Immunol* 2012;68:456-464.

- 6) Barnea ER. Applying embryo-derived immune tolerance to the treatment of immune disorders. *Ann N Y Acad Sci* 2007;1110:602-618.
- 7) Barnea ER. Insight into early pregnancy events: the emerging role of the embryo. *Am J Reprod Immunol* 2004;51:319-322.
- 8) Barnea ER, Kirk D, Ramu S, Rivnay B, Roussev R, Paidas MJ. PreImplantation Factor (PIF) orchestrates systemic anti-inflammatory response by immune cells: effect on peripheral blood mononuclear cells. *Am J Obstet Gynecol* 2012;207:313, e311-e311.
- 9) Barnea ER, Kirk D, Todorova K, McElhinney J, Hayrabedyan S, Fernandez N. PIF direct immune regulation: blocks mitogen-activated PBMCs proliferation, promotes TH2/TH1 bias, independent of Ca(2+). *Immunobiology* 2015;220:865-875.
- 10) Roussev RG, Dons'koi BV, Stamatkin C, Ramu S, Chernyshov VP, Coulam CB, et al. Preimplantation factor inhibits circulating natural killer cell cytotoxicity and reduces CD69 expression: implications for recurrent pregnancy loss therapy. *Reprod Biomed Online* 2013;26:79-87.
- 11) Tincani A, Bompane D, Danieli E, Doria A. Pregnancy, lupus and antiphospholipid syndrome (Hughes syndrome). *Lupus* 2006;15:156-160.
- 12) Borchers AT, Naguwa SM, Keen CL, Gershwin ME. The implications of autoimmunity and pregnancy. *J Autoimmun* 2010;34:J287-J299.
- 13) Barnea ER, Almogi-Hazan O, Or R, Mueller M, Ria F, Weiss L, et al. Immune regulatory and neuroprotective properties of preimplantation factor: from newborn to adult. *Pharmacol Ther* 2015;156:10-25.
- 14) Stamatkin CW, Roussev RG, Stout M, Absalon-Medina V, Ramu S, Goodman C, et al. PreImplantation Factor (PIF) correlates with early mammalian embryo development-bovine and murine models. *Reprod Biol Endocrinol* 2011;9:63.
- 15) Stamatkin CW, Roussev RG, Stout M, Coulam CB, Triche E, Godke RA, et al. Preimplantation factor negates embryo toxicity and promotes embryo development in culture. *Reprod Biomed Online* 2011;23:517-524.
- 16) Paidas MJ, Krikun G, Huang SJ, Jones R, Romano M, Annunziato J, et al. A genomic and proteomic investigation of the impact of preimplantation factor on human decidual cells. *Am J Obstet Gynecol* 2010;202:459, e451-e458.
- 17) Barnea ER, Kirk D, Paidas MJ. Preimplantation factor (PIF) promoting role in embryo implantation: increases endometrial integrin- α 2 β 3, amphiregulin and epiregulin while reducing betacellulin expression via MAPK in decidua. *Reprod Biol Endocrinol* 2012;10:50.
- 18) Duzjy CM, Paidas MJ, Jebailey L, Huang JS, Barnea ER. PreImplantation Factor (PIF*) promotes embryotrophic and neuroprotective decidual genes: effect negated by epidermal growth factor. *J Neurodev Disord* 2014;6:36.
- 19) Moindjie H, Santos ED, Loeuillet L, Gronier H, de Mazancourt P, Barnea ER, et al. Preimplantation factor (PIF) promotes human trophoblast invasion. *Biol Reprod* 2014;91:118.
- 20) Duzjy CM, Barnea ER, Li M, Huang SJ, Krikun G, Paidas MJ. Preimplantation factor promotes first trimester trophoblast invasion. *Am J Obstet Gynecol* 2010;203:402, e401-e404.
- 21) Di Simone N, Di Nicuolo F, Marana R, Castellani R, Ria F, Veglia M, et al. Synthetic PreImplantation Factor (PIF) prevents fetal loss by modulating LPS induced inflammatory response. *PLoS ONE* 2017;12: e0180642. <https://doi.org/10.1371/journal.pone.0180642>.
- 22) Goodale LF, Hayrabedran S, Todorova K, Roussev R, Ramu S, Stamatkin C, et al. PreImplantation factor (PIF) protects cultured embryos against oxidative stress: relevance for recurrent pregnancy loss (RPL) therapy. *Oncotarget* 2017;8:32419-32432.
- 23) Moindjie H, Dos Santos E, Gouesse R, Swierkowski-Blanchard N, Serazin V, Barnea ER, et al. PreImplantation Factor is an anti-apoptotic effector in human trophoblasts involving p53 signaling pathway. *Cell Death Disease* 2016;7:e2504.
- 24) Ramu S, Stamatkin C, Timms L, Ruble M, Roussev RG, Barnea ER. PreImplantation factor (PIF) detection in maternal circulation in early pregnancy correlates with live birth (bovine model). *Reprod Biol Endocrinol* 2013;11:105.
- 25) Azar Y, Shainer R, Almogi-Hazan O, Bringer R, Compton SR, Paidas MJ, et al. Preimplantation factor reduces graft-versus-host disease by regulating immune response and lowering oxidative stress (murine model). *Biol Blood Marrow Transplant* 2013;19:519-528.
- 26) Shainer R, Almogi-Hazan O, Berger A, Hinden L, Mueller M, Brodie C, et al. PreImplantation factor (PIF) therapy provides comprehensive protection against radiation induced pathologies. *Oncotarget* 2016;7:58975-58994.
- 27) Barnea ER, Hayrabedyan S, Todorova K, Almogi-Hazan O, Or R, Guingab J, et al. PreImplantation factor (PIF*) regulates systemic immunity and targets protective regulatory and cytoskeleton proteins. *Immunobiology* 2016;221:778-793.
- 28) Chen YC, Rivera J, Fitzgerald M, Hausding C, Ying YL, Wang X, et al. PreImplantation factor prevents atherosclerosis via its immunomodulatory effects without affecting serum lipids. *Thromb Haemost* 2016;115.
- 29) Barnea ER, Lubman DM, Liu YH, Absalon-Medina V, Hayrabedyan S, Todorova K, et al. Insight into PreImplantation Factor (PIF*) mechanism for embryo protection and development: target oxidative stress and protein misfolding (PDI and HSP) through essential RIKP binding site. *PLoS One* 2014;9:e100263.
- 30) Shainer R, Azar Y, Almogi-Hazan O, Bringer R, Compton SR, Paidas MJ, et al. Immune regulation and oxidative stress reduction by preimplantation factor following syngeneic or allogeneic bone marrow transplantation. *Conf Papers Med* 2013;2013: 1-8.
- 31) Almogi-Hazan O, Shainer R, Barnea ER, Or R. The role of nitric oxide toxicity and oxidative stress in graft vs host disease. In: *Oxidative Stress: Causes, Role in Diseases and Biological Effects*. Hauppauge, NY: Nova Science Publishers; 2014.
- 32) Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International autoimmune hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999;31:929-938.
- 33) Bauer LA. *Applied clinical pharmacokinetics*, 3rd edn. New York, NY: McGraw-Hill Medical; 2014.
- 34) Weiss L, Bernstein S, Jones R, Amunugama R, Krizman D, Jebailey L, et al. Preimplantation factor (PIF) analog prevents type I diabetes mellitus (T1DM) development by preserving pancreatic function in NOD mice. *Endocrine* 2011;40:41-54.
- 35) Migliara G, Mueller M, Piermattei A, Brodie C, Paidas MJ, Barnea ER, et al. PIF* promotes brain re-myelination locally while regulating systemic inflammation—clinically relevant multiple sclerosis M.smeagmatis model. *Oncotarget* 2017;8: 21834-21851.
- 36) Weiss L, Or R, Jones RC, Amunugama R, JeBailey L, Ramu S, et al. Preimplantation factor (PIF*) reverses neuroinflammation while promoting neural repair in EAE model. *J Neurol Sci* 2012;312:146-157.
- 37) Mueller M, Zhou J, Yang L, Gao Y, Wu F, Schoeberlein A, et al. PreImplantation factor promotes neuroprotection by targeting microRNA let-7. *Proc Natl Acad Sci U S A* 2014;111:13882-13887.