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Phase I Clinical Trial with a Novel Altered Peptide Ligand Derived from Human Heat-Shock Protein 60 for Treatment of Rheumatoid Arthritis: Safety, Pharmacokinetics and Preliminary Therapeutic Effects

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Abstract

Background: CIGB 814 is an Altered Peptide Ligand (APL) from a $CD4^+$ T-cell epitope of human heat shock protein 60 (HSP60), an auto-antigen involved in the pathogenesis of rheumatoid arthritis (RA). It induced mechanisms associated with restoration of peripheral tolerance in preclinical studies. This clinical trial was conducted to assess safety and pharmacokinetics (PK) of CIGB-814 in patients with RA.

Method: 20 patients with moderated active RA were included in an open label trial. Sequential dose-escalation of 1, 2.5 and 5 mg of CIGB-814 was studied. Consecutive groups of six, five and nine patients received a subcutaneous dose weekly of the peptide during the first month and one dose monthly during the next five months. Clinical response in patients was evaluated according to the American College of Rheumatology (ACR) and Disease Activity Score in 28 joints (DAS 28) criteria. Function and health-related quality of life, quantification of pro-inflammatory cytokines and radiographic changes in patients by magnetic resonance imaging (MRI) were also assessed.

Result: The treatment was well tolerated at all doses. Only mild events were observed. PK study showed that CIGB-814 reached the maximum concentration in plasma in 30 min and was cleared mostly after 4 h. CIGB-814 reduced disease activity and MRI score in patients. This effect was less marked with the dose of 5 mg. Five and eleven out of 18 patients achieved ACR 50 and ACR 70 respectively at the end of the treatment. In addition, patients showed decreases of DAS28 scores, during treatment and at the end of the follow-up. This therapy improved function and health-related quality of life of patients. CIGB-814 significantly decreased interleukin (IL)-17 in patients treated with 2.5 mg. Therapy with 1 mg and 2.5 mg of CIGB-814 led to significant reduction of interferon gamma (IFN-γ).

Conclusion: Phase I concluded showing safety of CIGB-814. The PK profile revealed that peptide is cleared from plasma very rapidly. Results indicated preliminary evidences of clinical efficacy and support further clinical investigation of this peptide for treatment of RA.

Trial registration: RPCEC00000238.

Keywords: APL; HSP60; Rheumatoid arthritis; Clinical trial; Safety

Abbreviations: AA: Adjuvant Induced Arthritis; AE: Adverse Event; ACR: American College of Rheumatology; APL: Altered Peptide Ligand; AUC: Area Under the Concentration Time Curve; CBC: Complete Blood Count; CIA: Collagen-Induced Arthritis; CIGB: Center for Genetic Engineering and Biotechnology, Cmax: Maximum Plasma Concentration; CRP: C-Reactive Protein; DAS28: Disease Activity Score In 28 Joints; DMARD: Disease-Modifying Anti-Rheumatic Drug; ELISA: Enzyme-Linked Immunosorbent Assay; ESR: Erythrocytes Sedimentation Rate; HAQ-CU: Cuban Adaptation of the Health Assessment Questionnaire-Disability Index; HSP60: Heat Shock Protein 60; IFN-γ: Interferon Gamma; IL: Interleukin; MCID: Minimal Clinically Important Difference; MRI: Magnetic Resonance Imaging; MTX: Methotrexate; NSAIDS: Non-steroidal Anti-Inflammatory Drugs; PBMCS: Peripheral Blood Mononuclear Cells; PK: Pharmacokinetics; RA: Rheumatoid Arthritis; RF: Rheumatoid Factor; SAMIS: Simplified Rheumatoid Arthritis Magnetic Resonance Imaging Score; SC: Subcutaneous;SF-36: Short Form 36 Health Survey; TCR: T-Cell Receptor; Treg: Regulatory T Cells; TNF-A: Tumor Necrosis Factor A; WBC: White Blood Cells Count; Anti-CCP:

Anticyclic Citrullinated Peptide; CDMARDS: Conventional Disease-Modifying Antirheumatic Drugs; DAS 28-ESR: Disease Activity Score For 28-Joint Counts Based on the ESR; ESR: Erythrocyte Sedimentation Rate; HAQ-CU: Cuban Adaptation of the Health Assessment Questionnaire-Disability Index.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by persistent inflammatory synovitis leading to various degrees of cartilage destruction, bone erosion, and finally deformity and loss of joint function. Although the etiology of RA is not totally understood, many studies have shown that T lymphocytes, macrophages, and proliferating synovial cells play a major role in the pathogenesis of this disease [1,2]. Methotrexate (MTX) is the standard therapy for RA, but despite the introduction of other disease-modifying anti-rheumatic drug (DMARD), only a fraction of RA cases achieved a complete remission [3].

Improved understanding of RA pathogenesis led to the development of several classes of biologic treatments. These therapies are an alternative for patients not responding to MTX or other DMARDs and constitute the best addition to the anti-rheumatic arsenal. Biologic agents are drugs targeting specific inflammatory cells, cellular interactions and cytokines that mediate RA-related tissue damage. Such treatments are designed to reduce signs and symptoms of RA and slow disease progression [4]. However, many patients have inadequate response to such therapies [5,6]. Drugs already approved by regulatory agencies remain insufficient for 40-50% of patients with RA [7].

In this context, other approaches need to be evaluated intensively, one of which is the induction of peripheral tolerance by antigen-specific therapy. This approach is aimed at eliminating T-cell clones that have escaped to the control mechanisms of peripheral tolerance [8]. The central role of T cells in the pathogenesis of RA is well established [9]. TH17 cell subset has been implicated in development and perpetuation of RA by secretion of pro-inflammatory cytokines such as: IL-17 and IL-21 which have pathogenic roles in this disease [10,11].

The potentialities of altered peptide ligands (APLs) as inductors of peripheral tolerance in experimental models have been reported by several authors [12-14]. APLs are similar to native epitopes but with one or several substitutions in the essential contact positions with T-Cell Receptor (TCR) or MHC class II molecules, modifying the cascade of necessary events for activation of T cells. These peptides can down-regulate the response of auto-reactive T cells by different mechanisms for controlling autoimmune diseases [13-15].

The selection of a specific auto-antigen for designing APLs is an essential point in this approach. HSP60 has been used in the induction of tolerance in autoimmune arthritis [16-18]. We previously predicted a novel $CD4^+$ T cell epitope from human HSP60 using bioinformatics tools [19]. In particular, one amino acid residue of the wild-type peptide was substituted for increasing its affinity to HLA class II molecules related to RA. According to preceding results, this APL (called previously APL-1 and here CIGB-814) increases the frequency of regulatory T cells (Treg) and their suppressive capacity against antigen responding effector $CD4^+$ T cells from RA patients. In addition, this peptide inhibits significantly IL-17 levels produced by effector $CD4^+$ T cells from peripheral blood mononuclear cells (PBMCs) of RA patients [19,20].

Furthermore, CIGB-814 reduced the course of adjuvant induced arthritis (AA) in Lewis rats [19]. Likewise, CIGB-814 efficiently inhibits collagen induced arthritis (CIA) in DBA/1 mice, associated with a decrease of tumor necrosis factor α (TNF-α) levels [21]. CIA is accompanied by a robust T- and B-cell response to type II collagen. This animal model has been widely used for testing new potential therapeutic agents for treatment of human RA [22]. CIGB-814 inhibits efficiently the course of arthritis in CIA, similar to MTX, which is the current standard treatment for RA. This fact suggests the therapeutic potential of CIGB-814 as a first-line therapeutic candidate for RA.

The primary intention of this study was to evaluate the safety of different doses during 28 weeks as well as determination of the PK profile of CIGB-814 in RA patients. In addition, this study explores the therapeutic potentials of this peptide.

Materials and Methods

Non-randomized and open phase-I clinical trial was designed to evaluate safety, tolerability, pharmacokinetics and preliminary therapeutics evidences of CIGB-814 in patients with RA. This study followed the principles of the Declaration of Helsinki for research in humans [23]. The Ethics and Scientifics Committees at each study site and the Cuban Regulatory Authority (CECMED) approved the protocol. All subjects provided theirs written informed consent. Patients were recruited from National Reference Center for Rheumatic Diseases, Havana. The therapy with CIGB-814 and clinical evaluations of patients took place at this Center. Imaging studies and Clinical Laboratory tests were performed at Medical and Surgical Research Center (CIMEQ), Havana. Pharmacokinetics and cytokines quantification were carried out at the Center for Genetic Engineering and Biotechnology (CIGB), Havana. This clinical trial was registered under number RPCEC00000238 at the Cuban Registry of Clinical Trials (www.registroclinico.sld.cu).

Patients

Patients from 19 to 65 years old with diagnosis of RA according to the criteria of the American College of Rheumatology/European League against Rheumatism [24] for at least 2 years were eligible for inclusion in the study. Enrolled patients had active disease despite treatment with at least one DMARD. Active disease was defined using DAS28-erythrocytes sedimentation rate (ESR) [25]. All patients received previous treatments with any DMARD, glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs). Elected patients were subjected to a washing period. This period oscillated between 15 days and one month in dependence of the previous treatments. Patients were ineligible if they had other rheumatic autoimmune diseases affecting the osteomioarticular system, other systemic autoimmune disorder or any overlap syndrome. Laboratory parameters within normal reference range were required. All patients had to be using a medically accepted form of contraception at the time of enrolment and had to continue its use through the follow up period. Patients taking drugs for concomitant diseases were required to keep them on stable doses prior to screening. Such stable doses had to be maintained throughout the study. Finally, twenty patients with moderate disease activity (3.2<DAS28<5.1) were enrolled in this study.

Study design

Study design was according to scale sequential doses of CIGB-814:1 mg, 2.5 mg and 5 mg. Patients were distributed into three groups corresponding to dose levels: 1 mg (six patients), 2.5 mg (five patients) and 5 mg (nine patients). Consecutive groups of patients received a subcutaneous dose weekly of the peptide during the first month and a monthly dose during the next five months. Patients were followed for six months after the last dose of the study.

The restriction for using DMARDs, glucocorticoids and NSAIDs was extended from the washout period, including the therapy phase and up to 3 weeks after the last CIGB-814 dose. These drugs could be administered if disease flares, according to the physician's criteria. Otherwise, only analgesics were permitted. The primary endpoints were safety and pharmacokinetic of CIGB-814. Clinical activity of the disease as well as function and health-related quality of life of patients were monitored at each visit.

Safety

Data from patients were collected at each visit and classified according to the Regulation 45/2007 from the Cuban Regulatory Authority: "Requirements for reporting of adverse events in current clinical trials, based on the WHO". This regulation agrees with criteria of National Cancer Institute Common Toxicity Criteria Adverse Event version 3.0 (National Cancer Institute, Frederick, MD, USA).

Pharmacokinetic assessments

PK study involved 19 out of 20 patients during the first CIGB-814 administration. Blood sampling included t=0 (before peptide shot) and t=0.5, 1, 1.5, 2, 4, 6, 8, 12, 18 and 24 h post administration. Plasma was obtained in EDTA vacationer tubes (Greiner BioOne, USA) by whole blood centrifugation at 2500 rpm for 15 min, divided into 500 µL aliquots and stored at −80°C. Samples were analysed by LC-MS/MS in Selected Reaction Monitoring (SRM) mode using a triple quadrupole mass spectrometer from Micromass. Each sample was analysed by triplicate. Briefly, 90 µL of thaw plasma were spiked with 10 µL of labelled peptide solution (to a final concentration of 12 ng/mL). Major proteins were precipitated with acetonitrile in a 2:1 ratio with plasma. Each sample was vortex mixed and centrifuged at 10,000 rpm for 5 min. Supernatant was spin dried for 1 h to remove acetonitrile and reconstituted in 100 µL of 5% LC elution solution. Main pharmacokinetic parameters were calculated with WinNonLin v2.1 software.

Clinical activity assessments

Exploratory analyses were conducted for evaluating the effect of CIGB-814 on clinical response variables, including: ACR20, ACR50 and ACR70 (20%, 50% and 70% improvement, respectively, in tender and swollen joints, as well as 20%, 50% and 70% improvement, respectively, in three of the other five ACR criteria for RA). DAS28 responses and ESR were also evaluated. These analyses were performed at baseline (T0) and at weeks 25, 28, 36 and 48. Complete Blood Count (CBC) and biochemical analysis of blood serum were obtained for patients at each visit.

Function and health-related quality of life assessments

Function and health-related quality of life were assessed in each patient using the variation from baseline and at weeks 12, 28, 36 and 48 by HAQ-CU (Cuban adaptation of the Health Assessment Questionnaire-Disability Index) [26] and Short Form 36 Health Survey (SF-36) questionnaire [27].

Cytokines assessments

Serum samples were obtained before treatment (T0) and at weeks 5, 13 and 25. IFN-γ and IL-17 concentrations in sera were assessed by ELISA (Quantikine®, R&D Systems, USA) according to the recommendations of the manufacturer, with lower limits of quantification of 15.6 pg/mL and 31.2 pg/mL respectively.

MRI assessments

MRI examination was performed before treatment (T0) and at weeks 28 and 48 using a clinical scanner (0.35 T Magnetom C, Siemens, Germany). A coil for children's knees was used. The same scanner and coil were used during all study. Pulse sequence parameters were adjusted and calibrated for this study. The sequences used were T1 Flash 3D with fat suppression (coronal and axial) with Echo Time (TE) and Repetition Time (TR) 50 ms and 17.9 ms, respectively. Coronal T2 Turbo short time inversion recovery (tirm) were performed with TR=3730 ms, TE=24 ms, TI 105 ms and slice thickness of 3-2.5 mm. Images were captured from dominant hand in each patient, since limb is most affected by mechanical effects associated with inflammatory process [28]. Erosions, bone edema and synovitis were quantified through Simplified Rheumatoid Arthritis Magnetic Resonance Imaging Score (SAMIS) [29].

Statistical analysis

All patients who received at least one dose of CIGB-814 were included in the safety, PK, clinical activity, and function and health-related quality of life. Adverse events (AEs), vital signs, laboratory tests and evidence of therapeutic effects were descriptively compared between baseline and data collected from patients at each planned visit according to the study design after beginning the CIGB-14 treatment with no formal statistical testing [30].

PK data were processed by non-compartmental analysis using WinNonLin Professional v2.1 software. PK parameters were reported as average per dose including standard deviation.

As part of the post hoc analyses, patients reporting improvements higher than minimal clinically important difference (MCID) for HAQ-CU and SF-36 were descriptively compared with baseline values in each visit using least squares mean change.

Cytokines data were analyzed using GraphPad Prism version 5.00 (GraphPad Sofware, San Diego California, USA). Samples were examined for normality and equal variance with Kolmogorov-Smirnov and Bartlett's tests, respectively. Results were expressed as mean ± standard deviation (SD) and differences during therapy were analysed with Kruskal Wallis and Dunn's post-test. P-values less than 0.05 were considered statistically significant.

MRI data were quantified according to SAMIS. This score was descriptively compared between T0 and at weeks 28 and 48. Number of areas corresponding to erosion, edema and synovitis were reported as average and percent per doses.

Results

Baseline characteristics and patient disposition

Twenty patients were enrolled in this study. Baseline demographics and characteristics were generally comparable between the three groups of treatment (Table 1). Age ranged from 38 to 62 years. Patients were predominantly women (85%) with moderate disease activity (100%) and a median duration of the disease of 8.5 years. All patients had received two or more DMARDs before enrolment (Table 1). Clinical status immediately before the first CIGB-814 dose was considered as baseline (T0).

Three patients dropped the study. One patient withdrew voluntarily and another one stopped due to allergy to paracetamol (cutaneous rash), both before therapy was completed. Another patient dropped voluntarily during follow-up stage (Table 2).

	1 mg (n=6)	2.5 mg (n=5)	5 mg (n=9)
Age, years*	52.0 ± 9.30	44.4 ± 6.27	50.9 ± 8.52
Gender, %female	100.00	100.00	66.66
Duration of RA, years	12.70 ± 5.68	6.00 ± 3.94	7.11 ± 8.95
Anti-CCP antibody†, %positive	60.00	60.00	62.50
RF‡, %positive	40.00	33.33	62.50
Methotrexate, % patients dose, mg/week	100.00 11.25	100.00 10.00	100.00 11.67
Concomitant cDMARDs for RA, %patients			
Hydroxychloroquine	66.66	40.00	33.33
Sulfasalazine	16.66	20.00	22.22
Prednisone use, %patients	100.00	100.00	100.00
Tender joints (28 count)	12.80 ± 5.63	10.40 ± 6.31	8.63 ± 2.67
Swollen joints (28 count)	8.40 ± 3.65	2.20 ± 1.30	3.63 ± 1.69
HAQ-CU§	1.12 ± 0.38	0.99 ± 0.44	2.01 ± 2.43
ESR, mm/h	24.60 ± 27.62	38.60 ± 19.83	40.50 ± 20.21
DAS28-ESR	4.34 ± 0.55	4.37 ± 0.64	4.62 ± 0.42

*Data reported as mean values ± SD unless otherwise indicated.

†Anti-CCP antibody positivity (>upper limit of normal (ULN)=25 U/mL).

‡RF positivity (>ULN=18 U/mL).

§Scores on the HAQ-CU range from 0 to 3, with higher scores indicating greater disability.

Table 1: Baseline characteristics and disease activity of patient populations.

	1 mg (n=6)	2.5 mg (n=5)	5 mg (n=9)
Adverse events (AEs)			
Serious AEs	0 (0)	0 (0)	0 (0)
Patients with ≥ 1 AEs	2 (33.3)	0 (0)	5 (55.6)
Medication changes due to AEs	0 (0)	0 (0)	0 (0)
Observed AEs			
Skin rash	1 (16.7)	0 (0)	0 (0)
Increased appetite	1 (16.7)	0 (0)	0 (0)
Pain at the injection site	1 (16.7)	0 (0)	4 (44.4)

Erythema at the injection site	0 (0)	0 (0)	1 (11.1)
Withdrawal's causes			
Volunteer	1 (16.7)*	0 (0)	1 (22.2)**
Paracetamol allergy	1 (16.7)***	0 (0)	0 (0)

*patient withdrawal at the follow-up stage

**patient withdrawal at week 3, after receiving 2 doses of CIGB-814

***patient withdrawal at week 5, after receiving 4 doses of CIGB-814

Table 2: Safety summary-number (%) of patients with adverse events, treatment alterations due to side effects and withdrawals during therapy.

Time (h)		**0**	**0.5**	**1**	**1.5**	**2**	**4**	**6**	**8**	**12**	**18**	**24**
Dose (mg)	1	0	12.4	6.7	3.1	2.2	0.1	0	0	0	0	0
	2.5	0	19.6	8.6	4.3	2.0	0	0	0	0	0	0
	5	0	29.9	14.3	9.2	5.8	1.8	0.4	0	0	0	0

Numbers correspond to the average for all patients analysed in each dose: 5, 5 and 9 patients for 1 mg, 2.5 mg and 5 mg respectively.

Table 3: Concentration (ng/mL) of CIGB-814 estimated at different times for each dose.

		1 mg (n=6)	**2.5 mg (n=5)**	**5 mg (n=9)**	**Total n (%)**
Disease state according to DAS28-ESR*, n (%)					
Week 28	High disease activity (DAS28>5.1)	0 (0)	0 (0)	0 (0)	0 (0)
	Moderate disease activity (3.2>DAS28 ≤ 5.1)	0 (0)	2 (40)	4 (50)	6 (33.3)
	Low disease activity (2.6 ≥ DAS28 ≤ 3.2)	1 (20)	1 (20)	1 (12.5)	3 (16.7)
	Remission (DAS28<2.6)	4 (80)	2 (40)	3 (37.5)	9 (50)
Week 48	High disease activity (DAS28>5.1)	0 (0)	0 (0)	0 (0)	0 (0)
	Moderate disease activity (3.2>DAS28 ≤ 5.1)	0 (0)	0 (0)	1 (12.5)	1 (5.9)
	Low disease activity (2.6 ≥ DAS28 ≤ 3.2)	2 (50)	1 (20)	2 (25)	5 (29.4)
	Remission (DAS28<2.6)	2 (50)	4 (80)	5 (62.5)	11 (64.7)
Disease state according to ACR**, n (%)					
Week 28	ACR20	0 (0)	0 (0)	1 (12.5)	1 (5.6)
	ACR50	0 (0)	2 (40)	3 (37.5)	5 (27.8)
	ACR70	5 (100)	3 (60)	4 (50)	12 (66.67)
Week 48	ACR20	0 (0)	0 (0)	0 (0)	0 (0)
	ACR50	0 (0)	0 (0)	1 (14.3)	1 (5.9)
	ACR70	4 (100)	5 (100)	7 (87.5)	16 (94.1)

*DAS28-ESR=Disease Activity Score in 28 joints, erythrocyte sedimentation rate

**ACR20, ACR50 and ACR70=20%, 50% and 70% improvement, respectively, in tender or swollen joint counts, as well as 20%, 50% and 70% improvement, respectively, in three of the other five American College of Rheumatology criteria for rheumatoid arthritis.

Table 4: Clinical response.

Safety

Safety analysis was based on all subjects included in this study. None of the patients withdrew by safety reasons. No serious or moderate adverse events were reported during the therapy with CIGB-814 or the follow-up stage.

The most significant adverse event was pain at the site of inoculation, in five patients: a patient corresponding to the group inoculated with 1 mg and four patients corresponding to 5 mg group. A patient treated with this last dose had transient erythema at the inoculation site. A patient treated with 1 mg reported increased appetite (Table 2).

These adverse events were classified as non-serious with mild intensity and were reversible. None of them caused modification of the treatment.

Taking into consideration that CIGB-814 is an APL, which plays its role on immune function, we determined whether CIGB-814 therapy had an effect on the white blood cell count (WBC) and in particular, the lymphocyte population for all patients enrolled in the trial.

Doses (mg)	T0	Week 28	Week 48
1	18.8 ± 7.1	15.5 ± 7.4	16.5 ± 7.5
2.5	33.8 ± 5.3	30.6 ± 5.2	31.4 ± 5.0
5	37.4 ± 9.0	37.1 ± 9.5	36.1 ± 8.6

Table 5: SAMIS average by doses during the therapy.

Therapy with CIGB-814 does not modify WBC or CBC. Absolute lymphocyte counts were stable across the entire study, irrespective of the dose levels. This treatment did not modify the biochemical parameters during the full study.

	T0		Week 28			Week 48		
Radiologic events	No.	%	No.	%	Δ%	No.	%	Δ %
Erosion	17	100	17	100	0.0	17	100	0.0
Edema	10	58.8	8.0	47.1	11.7	5.0	29.4	29.4
Synovitis	16	94.1	9.0	52.9	41.2	14	82.4	11.7

Table 6: Number of radiological events identified in the patients.

Pharmacokinetics

PK parameters were calculated by Non Compartmental Analysis, assuming a phase of elimination between 1.5 and 2 h (Table 3). Overall, CIGB-814 exposure measured by maximum plasma concentration (Cmax) and area under the concentration time curve (AUC) indicated that these parameters were not dose-dependent, although both tended to increase with doses. CIGB-814 was cleared from plasma in 4 h for patients inoculated with 1 mg and 2.5 mg; and approximately in 6 h for patients treated with 5 mg. Cmax was reached at 0.5 h for the three doses, showing a wide dispersion, probably associated with the biological variability among patients and the non-normalized doses respect to the body weight (Table 3). (Journal of Pharmaceutical and Biomedical Analysis, in Press).

Clinical response

Preliminary efficacy analysis was performed, according to DAS28 and ACR criteria.

All patients began the treatment with moderate disease activity.

Two patients inoculated with 1 mg reduced theirs DAS-28 score to low activity, a week after receiving the first dose of CIGB-814. At week 28, four patients treated with this dose achieved remission and one patient had low activity according to this score. When these patients completed the follow-up stage, two continued in remission and two had low activity. At this stage, a patient left the study voluntarily (Table 4).

Patients treated with 2.5 mg had improvement of the disease. Two patients achieved remission and another one had low activity, three weeks after completed the therapy (week 28), while two patients did not reduced theirs DAS-28 score. However, at the end of the follow-up stage, four patients were in remission and another one had low activity (Table 4).

At week 28, three patients treated with the dose of 5 mg achieved remission and one patient had low disease activity. Four patients maintained moderate disease activity during treatment. Nevertheless, at the end of follow-up stage one of these patients achieved remission, two patients had low activity and only one continued with moderate disease activity. In summary, five patients treated with this dose were in remission, two with low activity and one with moderate activity at week 48. However, this patient with moderate activity achieved ACR 20 (Table 4).

All patients resume the therapy with MTX (7.5 mg orally) three weeks after the end of the treatment with CIGB-814, and no one needed prednisone. In all cases, the dose of MTX was lower than the one they had before enrolling this study (Table 1). At the end of the study, 11 out of 17 patients achieved DAS-28<2.6.

Using the EULAR28 measure of good or moderate response ([1]), all patients had good response to CIGB-814 therapy, since all of them had a reduction of DAS-28 greater than 1.2 at the end of treatment and during the follow-up stage (Figure 1A).

Improvements in the ACR responses were observed. All patients treated with 1 mg of CIGB-814 had ACR 70 at week 28 (Table 4). Patients evaluated preserved this ACR at the end of the follow-up stage.

At week 28, two and three patients treated with 2.5 mg achieved ACR 50 and 70, respectively. All patients achieved ACR 70 at the end of the follow-up stage (Table 4).

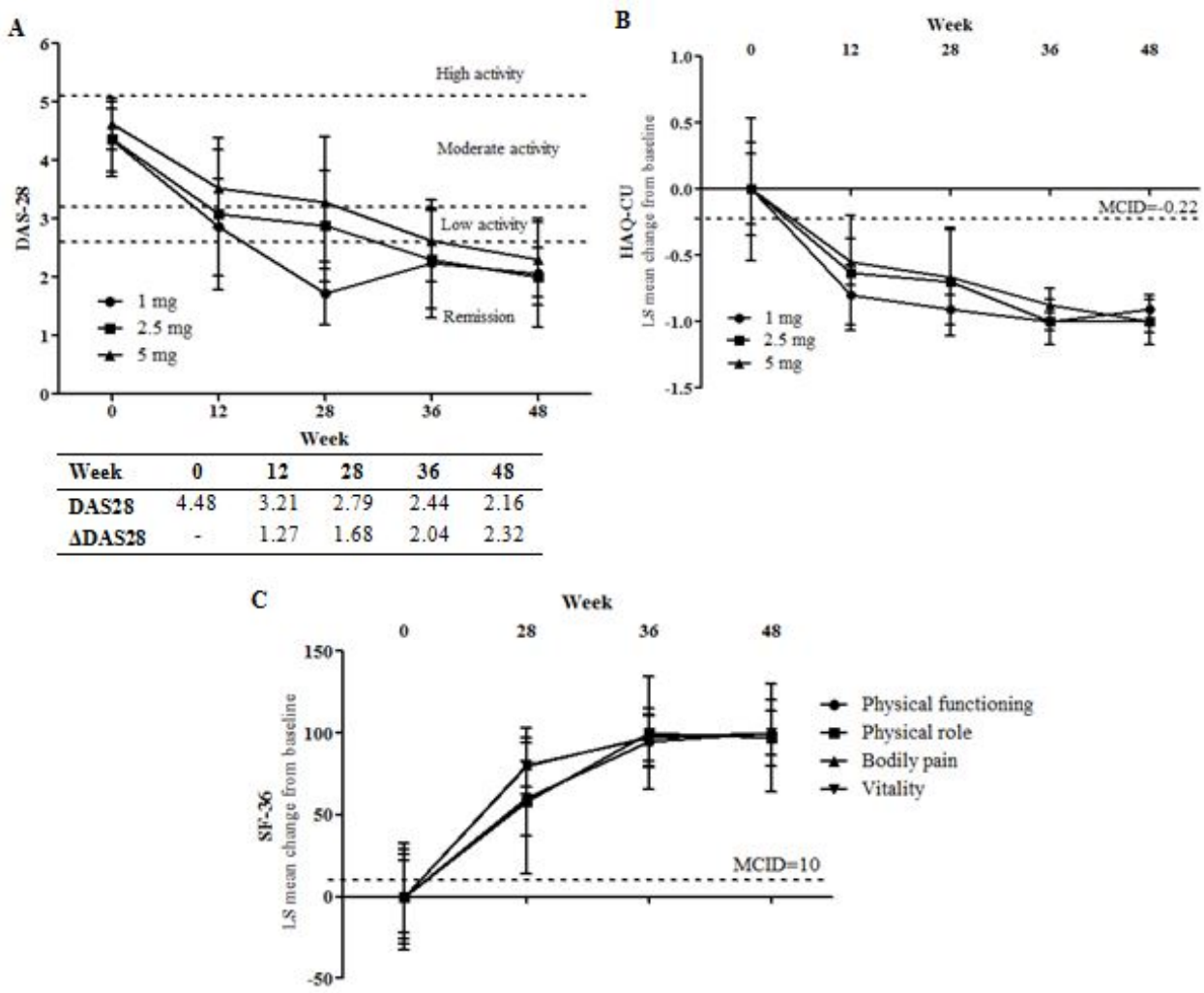
Only one patient treated with 5 mg of CIGB-814 did not achieve ACR at week 28. While three and four patients treated with this dose achieved ACR 50 and 70, respectively. At the end of the follow-up stage, one patient achieved ACR 50 and the others continued with ACR70 (Table 4).

Function and health-related quality of life

In this study, as shown in Figure 1B, all patients improved their functions and health-related quality of life. At beginning of the treatment, the mean HAQ-CU corresponded to moderate disability (1.07) (Table 1). During therapy with CIGB-814 and the follow-up

stage, this variable showed a gradual decrease to a mild disability (0.16) (Figure 1B).

Patients reported clinically meaningful improvements (least squares mean change from baseline) in HAQ-CU and SF-36 (Figures 1B and 1C).



Week	0	12	28	36	48
DAS28	4.48	3.21	2.79	2.44	2.16
ΔDAS28	-	1.27	1.68	2.04	2.32

Figure 1: (A) Effect of CIGB-814 on rates of remission (defined as a Disease Score in 28 joints (DAS28) of <2.6) at baseline (0) and at weeks: 12, 28, 36 and 48. Values are the mean ± standard deviation. Table above indicates the average of DAS28 at baseline (0) and at weeks: 12, 28, 36 and 48 and differences between averages of DAS28 at baseline and at weeks: 12, 28, 36 and 48. All patients had good response to CIGB-814 therapy, since all of them had a reduction of the DAS-28 greater than 1.2 at the end of treatment and during the follow-up stage. (B) Least squares mean change from baseline (0) in HAQ-CU (Cuban adaptation of the Health Assessment Questionnaire-Disability Index). All patients had a decrease of ≥ 0.22 points in the HAQ-CU, which is defined as the minimal clinically important difference (MCID) from baseline to week 28. (C) Least squares mean change from baseline (0) in SF-36. All patients reporting improvements of >10 points (MCID) in physical functioning, physical role, bodily pain and vitality domains.

Sixteen out of eighteen patients reported a decrease of ≥ 0.22 points in the HAQ-CU from baseline at week 12, which is defined as the minimal clinically important difference (MCID) [31,32]. All patients informed a decrease of >0.22 at week 28 (Figure 1B).

Assessment by SF-36 showed an improvement in most of the parameters and the best results were in physical role, physical function, bodily pain and vitality (Figure 1C).

Effect of CIGB-814 on pro-inflammatory cytokines

Levels of IFN-γ and IL-17 were investigated. Both cytokines were quantified in sera of patients before treatment and at weeks 5, 13 and 25 after beginning the therapy with CIGB-14. As shown in Figure 2A, therapy with 1 mg and 2.5 mg of CIGB-814 led to significant reduction of IFN-γ. However, treatment with 5 mg increased this cytokine at weeks 13 and 25 compared to week 5.

On the other hand, only therapy with 2.5 mg of CIGB-814 led to significant reduction of IL-17 (Figure 2B).

MRI assessments

Average of SAMIS by doses at T0, and weeks 28 and 48 are shown in table (Table 5). There was a decrease of approximately 3 points at the end of the treatment in patients treated with 1 and 2.5 mg. SAMIS increased at the end of the follow-up stage, but remaining below the value at T0. The decrease of SAMIS in patients treated with 5 mg was lower than in patients inoculated with 1 mg and 2.5 mg.

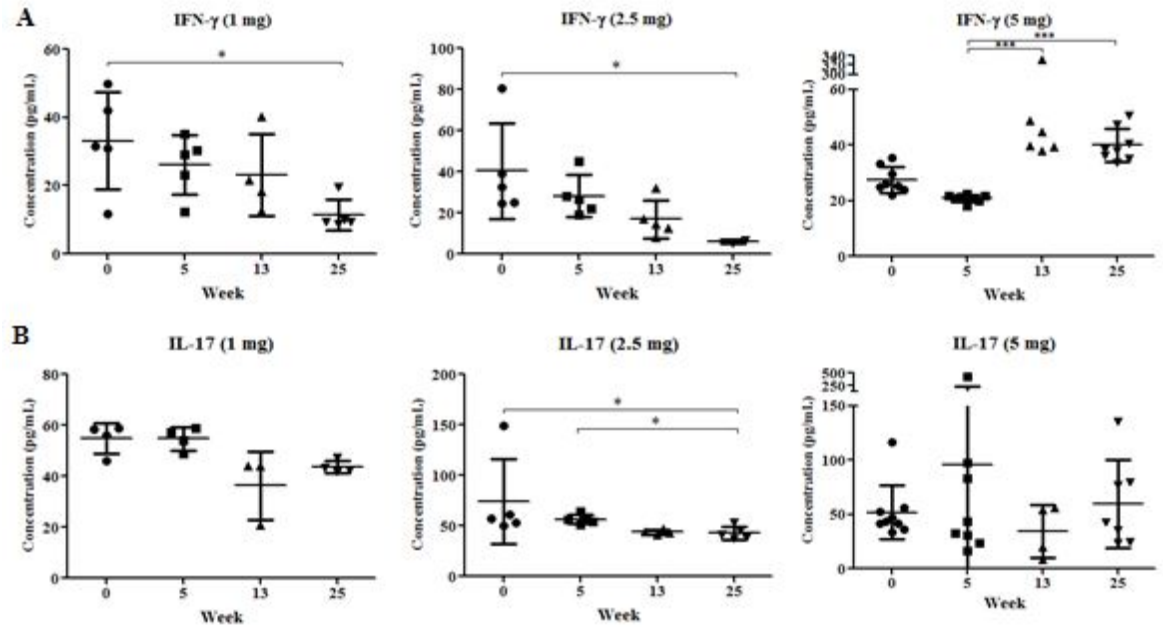


Figure 2: Effect of CIGB-814 on pro-inflammatory cytokines. (A) Levels of IFN-γ. (B) Levels of IL-17. Serum samples were obtained before treatment (0) and at weeks 5, 13 and 25. Cytokines concentrations in sera were assessed by ELISA. Concentrations of IFN-γ and Il-17 are expressed as mean+standard deviation and were analyzed using Kruskal-Wallis and Dunn post-test ($^{*}P<0.05$, $^{***}P<0.01$). These results are representative of three similar experiments.

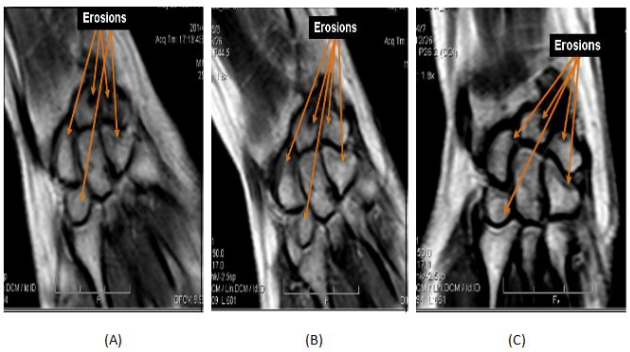


Figure 3: Representative images of a patient's hand (A) before starting treatment (B) at 28 weeks and (C) at 48 weeks (C). MR images show bone lesions consistent with erosions (oranges lines). Coronal orientation, Echo time/50 ms, Repetition time 17.9 ms.

Table 6 shows the specific changes associated with synovitis and edema during the therapy. New erosion sites were not observed and edema and synovitis decreased in the studied hand of patients. Representative images of a patient's hand before starting treatment and at weeks 28 and 48 are shown in Figure 3.

Discussion

A remarkable progress in the understanding of the mechanisms of rheumatoid arthritis has taken place in recent years and such understanding has been translated into novel anti-cytokines approaches. However, these therapies have some limitations: induction of adverse events, approximately 40% of patients do not respond and the high cost of treatment [33]. This has led several groups to focus on other therapeutic approaches.

Antigen-specific immunomodulation provides a tool for inducing peripheral tolerance to pathogenic T-cell clones involving simultaneous activation of multiple regulatory mechanisms. APLs are an option for this approach. Conceptually, such peptide mediated therapeutic intervention is based on modulation of antigen specific T cells and therefore lower toxicity is expected, compared to agents targeting broadly active inflammatory cytokines [34].

We previously reported that CIGB-814 (APL-1) increases the frequency of $CD4^{+}CD25$ high$FoxP3^{+}$ Treg in *ex vivo* assays using PBMC or SFMC from RA patients. Congruently, CIGB-814 enhanced the suppressive functions of $CD4^{+}CD25$ highCD127-against antigen responsive $CD4^{+}CD25^{-}CD127^{+}$ T cells (Teff cells), whereas activated Teff cells produce less IL-17. In addition, this peptide efficiently inhibited the course of adjuvant-induced arthritis (AA) in Lewis rats and collagen induced arthritis (CIA) in DBA-1 mice [19-21].

In the present study, safety and pharmacokinetics of CIGB-814 were evaluated in patients with RA. The peptide was very well tolerated. The adverse events observed were mild and reversible, mainly redness and pain at the site of inoculation. Furthermore, patients did not have signs or symptoms, which could be interpreted as immunosuppression, during the therapy and the follow-up stage. This fact and low toxicity of CIGB-814 is according to the therapeutic approach. In particular, molecular mechanism of CIGB-814 in preclinical studies has been associated with increase of Treg and decrease of TNF-α and IL-17, but without decreasing the percentage of Teff cells, suggesting a decrease of chronic inflammation associated with regulation of the immune system [19-21].

Pharmacokinetic profile of CIGB-814 is in correspondence with its chemical nature. The maximum concentration in blood occurred at 30 min with fast elimination (Table 3). Previously, we studied bioavailability and bio-distribution of CIGB-814 in Lewis rats. Peptide was localized mainly in the gastrointestinal tract and lymph nodes after 4 h of inoculation. The maximum concentration was reached between 30 min to 1 h in rats (unpublished results). We may expect similar behaviour for humans and the peptide may go to similar organs, which would be beneficial for immunomodulation therapies.

On the other hand, we found that mice receiving only a subcutaneous dose of CIGB-814 significantly increased Treg in the draining lymph nodes four days after inoculation [19].

Probably, CIGB-814 was arrested in draining lymph nodes of patients as happened in animals. Possibly the peptide is retained in the lymph nodes, presented by the Antigen Presenting Cells (APC) to the T naive lymphocytes and modifying the signalling cascade of activation. It may be evidenced by modification of the levels of inflammatory cytokines. In this study we found that CIGB-814 therapy with 1 and 2.5 mg decreases the levels of INF-γ.

IFN-γ characterizes TH1 response pattern, which is determinant in the pathogenesis of RA [35]. Different phenotypes of TH cells do not constitute terminal differentiation patterns consequently, they are partially differentiated populations with plasticity in their polarization [36,37]. APC can direct the differentiation of $CD4^+$ T cells against an antigen determined as an APL, through different signals such as secretion of cytokines.

However, treatment with 5 mg increased IFN-γ at weeks 13 and 25 compared to week 5. This result suggests that this approach is dependent of the CIGB-814 dose and low doses may favour the induction of tolerance.

On the other hand, CIGB-814 significantly decreases IL-17 in patients treated with 2.5 mg. IL-17 initiates the inflammatory response and promotes cartilage and bone damages in RA. IL-17 induces the production of other inflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-23 by synovial fibroblasts, monocytes and macrophages, enhancing inflammation and development of TH17 [38]. In addition, IL-17 increases the production of matrix metalloproteinase and nitric oxide in chondrocytes and osteoblasts, which lead to degradation of cartilage and bone [39].

Reduction of IL-17 and IFN-γ were in correspondence with the clinical response of patients. Preliminary evidences of decreased clinical activity were observed for the three doses measured by reduction of DAS28 and achievement of ACR20, ACR50, and ACR70 responses. Patients showed decreases of DAS28 scores, during treatment and the follow-up stage. All patients had good response to CIGB-814 therapy according EULAR28, since all of them had a reduction of DAS28 greater than 1.2 at the end of treatment and during the follow-up stage [24].

ACR50 and ACR70 response rates were observed in 27.8% and 66.7% of patients, when they finished the treatment. Additionally, preliminary evidence of therapeutic effectiveness were observed in 94% of patients maintaining ACR70 score up to 6 months after receiving the last dose of the drug.

All patients resume the treatment with 7.5 mg of oral MTX three weeks after the end of the study with CIGB-814, and no one needed prednisone. Notice that in all cases, the dose of MTX was lower than the one they had before enrolling this study (Table 1). It is interesting that patients treated with 1 and 2.5 mg of CIGB-814 achieved ACR20, ACR50 and ACR 70 faster than patients inoculated with 5 mg.

According to ethical concerns, in the design of this study we consider the inclusion of MTX as a concomitant therapy at week 12 of treatment (or even before), depending on the clinical response of patients. However, it is remarkable that it was not necessary to include MTX or any other DMARD in any patient.

Patients were improving progressively. All patients achieved ACR20 at week 12. Six patients maintained a moderate activity according to DAS-28 at the end of treatment caused by ESR values, which although decreased, remained above normal values. However, five of these six patients achieved ACR 50 and the other one achieved ACR20 at the end of the therapy (week 28).

RA interferes with the daily activities of patients, so it is useful to evaluate the effect of treatment on those aspects of the most importance for patients. Here, we measured patients' function and health-related quality of life using the Cuban adaptation of the HAQ (HAQ-CU) [26] and generic health-related quality of life SF-36 questionnaires. CIGB-814 administration improved the HAQ-CU disability index and SF-36 physical component score. Most patients enrolled in this study improved their general behaviour and quotidian and work activities.

Therapy with CIGB-814 resulted in a sustained reduction in symptoms and signs of rheumatoid arthritis and increased the function of patients. Our results suggest that CIGB-814 reduced disability, even though it is generally accepted that two years of treatment is required to demonstrate prevention of disability [40].

MRI analysis corroborated the results obtained in the clinical evaluation. No new areas of bone erosion were found during the study. In addition, there was a reduction of edema and synovitis in the studied hand of patients. This reduction was less marked for the dose of 5 mg. Although we did not use contrast agent, it was possible to measure synovitis [41]. However, in future studies we will propose the usage of gadolinium, clinical scanners of 1.5 T and a wrist-specific coil to facilitate the analysis.

This is the first in-human dose escalation study of CIGB-184 to define a safety dose range and PK profile.

On the other hand, DAS-28 was measured using the ERS values of each patient. ERS values are slow to decrease, even when patients improve clinically. For this reason, we shall calculate DAS-28 using C-reactive protein (CRP) in future studies.

Larger studies will allow the assessment of the therapeutic effect of CIGB-814 on varied RA patient population. Phase II clinical trial to select an optimal dose, obtain stronger evidences of efficacy and to

continue evaluating the safety of CIGB-814 is in preparation. This study will be double blind and controlled. In addition, considering preclinical studies in which CIGB-814 increased Treg and its suppressive activity [19,20], we shall measure these cells in the treatment groups and the placebo-control arm.

Conclusion

This study characterizes the first clinical application of CIGB-814 in RA patients. The peptide was well tolerated in the three subcutaneous doses evaluated and displayed a satisfactory PK profile. CIGB-814 was cleared mostly after 4 h. CIGB-814 reduced disease activity and MRI score in patients. This effect was less marked with the dose of 5 mg. The treatment increased the quality of life of patients. Therapy with 1 and 2.5 mg decreases the levels of INF-γ. In addition, IL-17 is reduced in patients treated with 2.5 mg. These results suggest that CIGB-814 reduces pro-inflammatory responses in RA pathology through T-cell response modulation.

CIGB-814 inhibits efficiently the course of arthritis, similar to MTX, in preclinical CIA model. This result and those obtained in the present study suggest the therapeutic potential of CIGB-814 as a first-line therapeutic candidate for RA.

Further evaluation of CIGB-814 efficacy and selection of an optimal dose is necessary in larger clinical trials. A phase II study in RA patients to characterize therapeutic effect and safety of CIGB-814 plus MTX is in preparation.

Ethics Approval and Consent to Participate

The Ethics and Scientifics Committees at each study site approved the protocol: National Reference Center for Rheumatic Disease, Center for Genetic Engineering and Biotechnology, National Center for Clinical Trial, Center for Surgical Medical Research and Cuban Regulatory Authority (reference number: 05-017-12-B)

Consent for Publication

Not applicable

Availability of Data and Materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

DP was the principal investigator, participated in trial design, performed data review and reviewed the manuscript. JG, AML, YR, CM and OM participated in trial design and execution and reviewed the manuscript. EG, LP, LO and YM and CC performed MRI data analysis, participated in trial. OC performed cytokines data analysis, drafted figures and tables. AC, YR, VB and LJG determined the PK profile, data analysis, participated in trial execution. YB served as a medical monitor and performed data analysis. YA participated in trial design and performed data analysis. NL participated in trial execution. YC performed Complete Blood Count and biochemical analysis of patients. AH participated in trial execution. HG, EP, ML and OR produced the molecule under study. GP generated the molecule under study, drafted the manuscript. MCD generated the molecule under study, project leader, served as scientific advisor and drafted the manuscript. All authors revised and approved the final version of this manuscript.

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