RESEARCH

Interferon alpha-2b and ribavirin as combined therapy for chronic hepatitis C in Cuba: National Program

✍ Hugo Nodarse-Cuní[1], Enrique Arús-Soler[2], Luis L Rivera-Reimón[3], Marlén Pérez-Lorenzo[4], Marcia Samada-Suárez[5], Waldo O García-Ferrera[6], Yoan A Sánchez-Rodríguez[2], Daymela Pupo-Oliveros[6], Angel Obregón-Moreno[7], Ivis Umpierre-García[8], Orelvis Martínez-Martínez[9], Antonio Moret-Pérez[10], Yaimé González-González[11], Yuliet Bermúdez-Hernández[12], Elizeth García-Iglesias[1], Odalys C Lazo-Diago[1], Pedro López-Saura[1], Cuban Group for the study of liver diseases[13]

[1] División de Ensayos Clínicos, Centro de Investigaciones Biológicas, CIB Calle 134, No. 152 e/ 21 y 23, Cubanacán, Playa, AP 6332, La Habana, Cuba
[2] Instituto Nacional de Gastroenterología; [3] Hospital Militar Dr. Carlos J Finlay; [4] Hospital Militar Central Dr. Luis Díaz Soto; [5] Centro de Investigaciones Médico-Quirúrgicas, Cimeq y [6] Hospital Calixto García, La Habana, Cuba
[7] Hospital Clínico-Quirúrgico Provincial Abel Santamaría Cuadrado, Pinar del Río, Cuba
[8] Hospital Militar Docente Dr. Mario Muñoz Monroy, Matanzas, Cuba
[9] Hospital Gustavo Aldereguía, Cienfuegos, Cuba
[10] Hospital Provincial Universitario Arnaldo Milián Castro, Villa Clara, Cuba
[11] Centro de Inmunoensayo, CIE, La Habana, Cuba
[12] Centro Nacional Coordinador de Ensayos Clínicos, Cencec, La Habana, Cuba
[13] See the Supplementary information for details of the Cuban Group for the study of liver diseases
E-mail: hugo.nodarse@cigb.edu.cu

ABSTRACT

The treatment of chronic hepatitis C with interferon alpha (IFN-α) is widely used. However, the relapse rate is high, and sustained response is only in 10-20%. A combined treatment based on the synergic antiviral effects described for IFN-α and ribavirin was used as a National Program in Cuba. The study enrolled 357 patients treated during 48 weeks with an injection of IFN-α, 3 times weekly, combined with oral ribavirin in daily doses, the doses adjusted to body weight. Sustained virological response was the efficacy end point, supported by biochemical and histological changes. Normalization in transaminase levels occurs in 60.5% of patients after the first 4 weeks, 71.4% at 26 weeks and 60.2% at the end of treatment. In similar moments, the viral load was undetectable in 42.9%, 42.6% and 37.0% respectively. The implementation of this National Program led to 49.0% and 29.7% of biochemical and virological sustained response respectively. A histological improvement was observed in 53.5% of evaluated patients. The treatment was well tolerated and almost all adverse reactions were attributable to IFN-α. The main adverse reports were: anemia, leucopenia, asthenia, fever, headache, arthralgias, anorexia and myalgia. Anti-interferon antibodies were developed in 38 patients, in 3 of them as neutralizing of antiviral activity. These results confirm the efficacy and security profile of both drugs as combined therapy for the chronic hepatitis C and represent the first clinical data generated from its extensive use in the Cuban general population. The virological response was in agreement with international reports for populations with similar characteristics.

Keywords: liver disease, treatment, interferon, ribavirin, clinical trial

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RESUMEN

Interferón alfa-2b y ribavirina como tratamiento combinado para la hepatitis C crónica en Cuba: Programa Nacional. El porcentaje de recaída tras el tratamiento de la hepatitis C crónica con interferón alfa (IFN-α) es elevado y la respuesta sostenida oscila entre el 10 y el 20%. El efecto antiviral sinérgico entre el IFN-α y la ribavirina fue la base para instaurar el tratamiento combinado mediante un programa nacional en Cuba. Se escogieron 357 pacientes que durante 48 semanas recibieron IFN-α, 3 veces por semana, y varias dosis de ribavirina según el peso corporal. La respuesta virológica sostenida fue la variable principal de eficacia, apoyada en las variaciones bioquímicas e histológicas. Tras las primeras 4 semanas, el 60.5% de los pacientes tenía la transaminasa normal; 71.4% en la semana 26, y 60.2% al concluir el tratamiento. En iguales momentos, la carga viral fue indetectable en 42.9, 42.6 y 37%, respectivamente. En 7 años de ejecución, se registraron 49 y 29.7% de respuesta bioquímica y virológica sostenida. Hubo una mejoría histológica en el 53.3% de estos pacientes. El tratamiento fue bien tolerado y casi todas las reacciones adversas se atribuyeron al IFN-α. Las principales fueron anemia, leucopenia, astenia, fiebre, cefalea, artralgias, anorexia y mialgias. En 38 pacientes se formaron anticuerpos anti- IFN-α y en 3 pacientes neutralizaron la actividad antiviral. Los resultados confirman la eficacia y el perfil de seguridad de esa combinación para tratar la hepatitis C crónica. Son los primeros datos clínicos tras su uso en la población cubana. La respuesta virológica coincide con la descrita en otras poblaciones con características similares.

Palabras clave: enfermedad hepática, tratamiento, interferón, ribavirina, ensayo clínico

✍ Corresponding author

Introduction

Hepatitis C virus (HCV) is the major causative agent of non-A, non-B hepatitis, this hepatic disease being characterized by elevated levels of the alanine-aminotransferase (ALT) up to two-fold the normal value and the presence of particles of viral RNA (HCV RNA) in serum of the patient [1, 2]. Hepatitis C remained very often as a clinically silent infection, usually and incidentally detected at the time of routine health insurance examination or when donating blood [3, 4].

HCV infection becomes chronic in about 85% of individuals with persistence of HCV RNA in serum and persistently or intermittently raised concentrations of ALT [5]. The global prevalence of HCV is estimated to average 3%, ranging from 0.1 to 10% in different countries [6]. In Africa and the Middle East is considerably higher while in Latin America is among middle and low, in particular Cuba reporting up to 1% of incidence [7, 8]. HCV is the first indication for liver transplantation; natural history suggests cirrhosis in a term of 20 years of the infection [9].

Since 1991, recombinant interferon alpha (IFN-α) treatment is an approved therapy for patients with chronic hepatitis C infection in the Food and Drug Administration (FDA) of USA [10, 11]. Standard therapeutic regimen consists in 3×10^6 IU of IFN-α three times weekly during 48 weeks with 15% sustained response. The main weakness is the high probability that the patients experience a viral recurrence or a relapse of the viral load after concluded the treatment [12, 13].

The use of antiviral drugs is one of the most favorable options to increase the response and to diminish the frequency of relapses. Ribavirin, a synthetic guanosine nucleoside analogue, decreases serum transaminase and contributed to some histological improvement, but has no effect on serum HCV RNA concentration and none of the cases overcame the results achieved with IFN-α monotherapy [14, 15].

The advance of the therapeutic studies has confirmed the strategy dedicated to commend the treatment of chronic hepatitis C towards the initial and sustained responses. On this purpose, combinations of immunomodulatory and antiviral drugs offer the best benefits, IFN-α and ribavirin as the most prominent [16]. Compared to standard therapy, IFN-α and ribavirin combined treatment doubles the response rate for all measures of efficacy always with an acceptable safety profile [17, 18]. In June 1998 after the results reached in the treatment of the chronic hepatitis C using IFN-α and ribavirin, the FDA included this combination among the therapies approved. Further, the National Institutes of Health Consensus Statement on Management of HCV infection in 2002 declared that 40-46% of patients reached sustained virological response and only 10-16% with adverse events causing withdrawal of treatment [19].

Several clinical trials have been made in Cuba using IFN-α2b monotherapy in patients with chronic hepatitis C. The results show that the control patients failed to respond to treatment, while 31% of the treated patients reached normal levels of transaminases [20, 21]. Another important experience was in the treatment of patients with acute hepatitis C, because 54% of ALT normalization and 85% of histological improvement was obtained [22].

A preliminary randomized, double-blind and placebo controlled clinical trial using recombinant IFN-α2b and ribavirin combined therapy was executed. This trial involved 47 chronic HCV patients and demonstrates 65% of HCV RNA clearance, 72% of ALT normalization and 73% of histological improvement [23].

In 2001, the Cuban National Program with IFNα2b and ribavirin for the treatment of chronic HCV started. The purpose was to extend this alternative for the whole population of patients with these diagnoses in the country.

Materials and methods

Patients

Individuals over 18 years of age were eligible for the study. As inclusion criteria, the positive results were established from a commercial available 3rd generation immunoenzimatic assay (UMELISA® HCV, Immunoassay Center, Havana, Cuba), and from a validated commercial qualitative detection assay of HCV RNA in serum (UMELOSA® HCV CUALITATIVO, Immunoassay Center, Havana, Cuba) [24]. All patients had histological confirmation of liver damage according to Knodell index [25]. The exclusion criteria were pregnancy, non-compensated chronic diseases; hemoglobin values lower than 11.0 g/dL in women or 12.0 g/dL in man. A total of 357 patients were included, 239 naïve and 118 who relapsed after IFN-α monotherapy.

Study design

This multicenter post-commercialization trial was initiated in November 2001 as a National Program. The consecutive inclusion of patients was extended until September 2007 and considered nation-wide active in Cuba, with the participation of 25 health institutions. The corresponding Ethics Committee approved the trial. The study was carried out according to the ethical principles contained in the Helsinki Declaration and following the Good Clinical Practices. The participation of all the subjects was totally voluntary as expressed by signing the prior written consent.

All patients received subcutaneous or intramuscular IFN-α2b (Heberon ® alfa R, Heber Biotec S.A., Cuba) 3 times per week and ribavirin (Novatec, Cuba) daily during 48 weeks. Ribavirin was given orally at a dose of 1000 mg per day (for body weight lower than 75 kg) or 1200 mg per day (for body weight above or equal to 75 kg). The use of antipyretic medications was oriented to diminish the intensity of adverse events inherent to IFN-α. The treatment was ambulatory. When the treatment was finished, a follow-up period of 24 weeks was established.

Assessment and end point

The viral genotype was determined with the Linear Array HCV Genotyping Test and viral load quantified using COBAS AmpliPrep/COBAS TaqMan HCV Test (lower detection limit of 15 IU/mL), both products from Roche Molecular Systems Inc. Clinical and

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safety evaluations of patients were carried out monthly during active therapy and follow-up periods.

The principal end point for efficacy analysis was the clearance of viral load. The biochemical (ALT value) and histological evaluations (by Knodell index at the end of follow-up) were secondary variables. The treatment response was attributable to patients with a negative detection of viral particle in serum after the last week of treatment and considered as sustained when the undetectable levels were maintained for six months later. The lower detection limit with UMELOSA® HCV CUALITATIVO was 101.7 IU/mL of HCV RNA [24].

The presence of anti-IFN-α antibodies was monitored every three months during treatment, using a sandwich capture ELISA system described by González-Cabañas *et al*. [26]. The procedure involved incubation of the samples on polystyrene plates coated with IFN-α and the later addition of a protein A-peroxidase conjugate. In the positive cases, their capacity to neutralize the antiviral activity of IFN-α was subsequently investigated. Both systems were developed and validated by the Center for Genetic Engineering and Biotechnology (CIGB) of Havana, Cuba.

Monthly clinical evaluations identified adverse events of treatment and were related to hematological and biochemical parameters using standard clinical laboratory procedures. All efficacy and security analyses were made by 'intention to treat' and descriptive statistics were applied.

Results

Tables 1 and 2 show the demographic variables and baseline disease characteristics among the HCV patients studied. This information was comparable in both groups of patients and remained unaffected by the multicenter design used. These data are consistent with the typical profile of any population of patients with chronic hepatitis C. The demographic findings are in agreement with the distributions for gender and skin color described for Cuban populations in the statistical annual report from the Ministry of Public Health [27].

Treatment started in 346 of the originally included 357 patients (96.9%), of them 274 (76.8%) completing the 48 weeks of therapy and 270 (75.6%) finishing the additional six months of follow-up. A definitive withdrawal of therapy occurred in 72 patients (20.2%), only 24 of these discontinuations of treatment being motivated by intense adverse events and other diseases related to the patients, the rest were voluntary decisions.

After 4 weeks of treatment, 216 patients (60.5%) showed a normal value of ALT and 42.9% of evaluated patient's without detectable viral particle in serum. The evaluation after 26 weeks of therapy increased the biochemical complete response in up to 255 patients (71.4%) and 152 patients (42.6%) showed clearance of viral load. For the group of patients treated for 48 weeks, ALT levels were normalized in 215 (60.2%) and viral particle were undetectable in 132 (37.0%).

The sustained evaluation after 78 weeks showed 175 (49.0%) patients with stable normal ALT value and 106 (29.7%) with sustained clearance of viral load; of them, 75 (42.9%) and 39 (36.8%) agreed to receive a liver biopsy, respectively. This tendency among HCV patients, not to attend for follow-up liver biopsy (the most invasive test they can be subjected to) if a virological response is attained during treatment, is explained by their perception of being benefited by treatment. By the contrary, the patients showing only biochemical response knew it does not indicate disease control as the virological response does, then attending more frequently for biopsy to confirm treatment benefits.

Table 1. Demographic characteristics of HCV patients included in the study (n = 357)

	Gender (%)		Skin color (%)		Age
	Male	Female	White	Nonwhite	(mean ± SD)
Naïve	114 (47.7)	125 (52.3)	185 (77.4)	54 (22.6)	44.2 ± 12.3
Nonresponder or relapse	59 (50.0)	59 (50.0)	93 (78.8)	25 (21.2)	47.3 ± 9.9
Total	173 (48.5)	184 (51.5)	278 (77.9)	79 (22.1)	45.3 ± 11.6

SD: standard deviation

There were 129 patients biopsied in total, including 15 who consented in spite of having neither biochemical nor virological response. Their histological evaluation at the end of follow-up evidenced an improvement in 69 (53.5%) including remission of the lesion in 15 (11.6%), according to the Knodell index. The results from all responses' variables were stronger for previously untreated patients compared to nonresponders or relapse to IFN-α monotherapy (Table 3).

Adherence to treatment made more evident the possibility of achieving clearance of viral particles and ALT normalization. In the case of histological evaluation after paired liver biopsies examination, improvements in terms of reducing both necroinflammatory activity and fibrosis grade could be obtained, with or without clearance of viral particle from serum (Table 4).

The viral genotype was determined with the Linear Array HCV Genotyping Test in 33 patients, finding genotype 1a in two patients and 1b in the other 31. The virological response in this group only occurred in 6 patients with genotype 1b. Baseline viral load and its variation during treatment and follow-up were quantified in 38 patients using the COBAS AmpliPrep/ COBAS TaqMan HCV Test, its lower detection limit of 15 IU/mL, and very good coincidence between the clearance in more than 2 log of viral loads and the qualitative result used as end point were attained.

The presence of antibodies against IFN-α was tested in sera of 206 patients and found positive in 38 of them, for an 18.4% frequency of appearance. Neutralizing antibodies against the IFN-α antiviral activity were detected in three patients, representing

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Table 2. Baseline disease information of HCV patients studied

Hepatic fibrosis	Naïve (%)	Nonresponder or relapse (%)	Total (%)
Fibrosis expansion of some portal areas, with or without short fibrous septa	48 (20.0)	27 (22.9)	75 (21.0)
Fibrosis expansion of most portal areas, with or without short fibrous septa	11 (4.6)	12 (10.2)	23 (6.4)
Fibrosis expansion of most portal areas, with occasional portal to portal bridging	41 (17.2)	18 (15.3)	59 (16.5)
Fibrosis expansion of portal areas, with marked bridging (portal to portal as well as portal to central)	22 (9.2)	14 (11.9)	36 (10.0)

Table 3. Histological response by Knodell index and fibrosis grade among treated patients*

Parameter	Knodell index			Fibrosis grade		
	Naïve (%)	Nonresponder or relapse (%)	Total (%)	Naïve (%)	Nonresponder or relapse (%)	Total (%)
Fibrosis remained null	-	-	-	21 (26.9)	12 (23.5)	33 (25.6)
Remission	9 (11.5)	6 (11.8)	15 (11.6)	14 (17.9)	10 (19.6)	24 (18.6)
Improvement	33 (42.3)	21 (41.1)	54 (41.9)	8 (10.3)	5 (9.8)	13 (10.1)
Stabilization	12 (15.4)	5 (9.8)	17 (13.2)	14 (17.9)	10 (19.6)	24 (18.6)
Worsening	24 (30.8)	19 (37.3)	43 (33.3)	18 (23.1)	9 (17.7)	27 (20.9)
Cirrhosis stable grade	-	-	-	3 (3.9)	5 (9.8)	8 (6.2)
Total	78 (60.5)	51 (39.5)	129	78 (60.5)	51 (39.5)	129

* Knodell index and fibrosis grade criteria: Remission: null score variation; Improvement: score reduction; Stabilization: no change in score; Worsening: increased score.

Table 4. Relation between variables of response

Variables		Biochemical (%)		Virological (%)		Histological (%)	
		Response	No response	Response	No response	Response	No response
Biochemical	Response	-	-	99 (56.6)	76 (43.4)	58 (77.3)	17 (22.7)
	No response	-	-	7 (7.4)	88 (92.6)	28 (51.9)	26 (48.1)
Virological	Response	99 (93.4)	7 (6.6)	-	-	33 (84.6)	6 (15.4)
	No response	76 (46.3)	88 (53.7)	-	-	53 (58.9)	37 (41.1)
Histological	Response	58 (67.4)	28 (32.6)	33 (38.4)	53 (61.6)	-	-
	No response	17 (39.5)	26 (60.5)	6 (14.0)	37 (86.0)	-	-

a 1.5% of antigenicity, all cases without virological or biochemical response to treatment.

Almost all adverse reactions reported were mild and attributable to IFN-α. The case of anemia could only be considered secondary to the use of ribavirin. The most frequent adverse events were detected by monitoring hematological parameters, with other important groups of reports referring influenza-like and musculoskeletal symptoms. There were fewer reports on dermatological, respiratory, psychiatric and gastrointestinal symptoms (Table 5).

Discussion

This National Program involved more than 300 Cuban patients in 5 years. There were no reports on similar series of consecutive patients treated for hepatitis C in Cuba. Previous clinical studies were limited to hospitals in Havana and application of IFN-α monotherapy [20-23]. The baseline disease characteristics and demographic variables described for treated patients met expectations for this type of disease, supporting the application of these results to the general hepatitis C Cuban population.

The occurrence of only 23.0% of definitive treatment interruption is a positive outcome in favor of adequate safety profile for combining both drugs, reinforced by the voluntary decision that justified more than half of these withdrawals. This finding differs from similar studies, where up to 90.0% of treatment withdrawals are related to the intensity of adverse events [28, 29].

Our results indicate that it is more probable to obtain benefit by combining IFN-α and ribavirin in chronic hepatitis C patients receiving it as first treatment than those with previous unresponsiveness or relapse to IFN-α monotherapy.

This conclusion does not introduce any new knowledge, due to numerous former reports by different hepatologists, making it an important prognosis therapeutic goal [30, 31].

Viral genotype and load determinations in a small group of patients represent two limitations of this work. However, treatment outcomes in genotyped patients coincided with those reported for similar populations and the higher frequency of genotype 1b was in agreement with data from other Latin American countries [32].

In the case of the histological evaluation, an interesting finding was the reduction of fibrosis scores, a condition usually considered irreversible. The global analysis including biochemical, virological and histological responses was useful to establish the impact of treatment on disease control. The acceptable

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Table 5. Safety profile of the study

Event	Naïve (%)	Nonresponder or relapse (%)	Total (%)
Anemia	177 (76.6)	90 (78.3)	267 (74.8)
Leucopenia	122 (52.8)	61 (53.0)	183 (52.9)
Asthenia	121 (52.4)	62 (53.9)	183 (52.9)
Fever	106 (45.9)	57 (49.6)	163 (47.1)
Headache	104 (45.0)	51 (44.3)	155 (44.8)
Arthralgia	90 (39.0)	50 (43.5)	140 (40.5)
Anorexia	82 (35.5)	52 (45.2)	134 (38.7)
Myalgia	79 (34.2)	40 (34.8)	119 (34.4)
Insomnia	51 (22.1)	26 (22.6)	77 (22.3)
Alopecia	44 (19.0)	28 (24.3)	72 (20.8)
Depression	46 (19.9)	23 (20.0)	69 (19.9)
Weight Loss	43 (18.6)	23 (20.0)	66 (19.1)
Dry mouth	44 (19.0)	21 (18.3)	65 (18.8)
Irritability	44 (19.1)	20 (17.4)	64 (18.5)
Chills	37 (16.0)	19 (16.5)	56 (16.2)
Neutropenia	35 (15.2)	16 (13.9)	51 (14.7)
Thrombocytopenia	29 (12.6)	17 (14.8)	46 (13.3)
Dizziness	28 (12.1)	13 (11.3)	41 (11.8)
Nauseas	28 (12.1)	9 (7.8)	37 (10.7)

coincidence of these three parameters indicates that using this combination leads to benefits that can be obtained, even, in the long term. This finding is consistent with current therapeutic expectations for chronic hepatitis C [33].

The safety profile of the study is coherent with those of similar trials reported for the drug and dosage used [34, 35]. It was confirmed that the addition of ribavirin only negatively impacted on the number of patients with anemia and its severity. As similar studies, the reduction of ribavirin dose attained to counteract anemia did not affect the therapeutic efficacy to achieve sustained viral clearance [36, 37]. The 1.5% of antigenicity found with Heberon® alfa R was lower than the 4.1% reported for Intron A [38] and 32.5% for Roferon A [39]. This is an advantage for the most successful clinical application of recombinant products [40, 41].

The national extension of IFN-α and ribavirin therapy was performed with flexible inclusion criteria for the entire Cuban population with hepatitis C. Under this condition, we expected lower sustained virological response compared to the 65% reported by Galbán and Vega [23] in the preliminary study with a small pre-selected population.

In summary, the efficacy and adequate security profiles obtained in this work for the combination of IFN-α and ribavirin are in agreement, as expected, with the international therapeutic consensus for the hepatitis C. The initiation of this program and subsequent generalization of this treatment alternative to the National Health System led to improved disease control, also increasing in 13% the sustained response compared to IFN-α monotherapy.

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Declaration of interest

Hugo Nodarse-Cuní, Elizeth García-Iglesias, Odalys C Lazo-Diago and Pedro López-Saura are employees of the Center for Biological Research, which is part of the CIGB, where Heberon ® alfa R is produced. The rest of the authors have no conflict of interests.

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Members of the Cuban group for the study of liver diseases

Note: The number of members per institution are declared in parenthesis. (*) Steering and Data Quality Committee.

Patient recruitment, treatment and follow-up (number of patients included at each site)

Instituto Nacional de Gastroenterología, La Habana (135)
Enrique Arús Soler (*), Yoan Antonio Sánchez Rodríguez, Eduardo Vilar-Gómez, Bienvenido Grá Oramas (*), Héctor Vega Sánchez, Ricardo Pérez Plasencia,
Sara Nápoles Aguilar

Hospital Universitario General Calixto García (40)
Waldo Orlando García-Ferrera (*), Daymela Pupo Oliveros, Jorge Luis García Menocal, Georgina Pérez, Susana Martínez Beatón, Editha Hydes

Hospital Militar Dr. Carlos J. Finlay, La Habana (34)
Luis Lázaro Rivera Reimón (*), Amada Palomino, Rosa Campos, Maylin Isabel Alonso, Lázara Dueñas Hernández

Hospital Militar Central Dr. Luis Díaz Soto, La Habana (31)
Marlén Pérez Lorenzo (*), Teresita Montero-González, Sonia Rodríguez Lasso

Hospital Clínico Quirúrgico Provincial Abel Santamaría Cuadrado, Pinar del Río (30)
Jorge Luis Álvarez Álvarez, Angel Obregón Moreno, Guillermo Fernández Maqueira, Gladys Cirión Martínez, José Antonio Gato

Centro de Investigaciones Médico-Quirúrgicas, La Habana (12)
Marcia Samada Suárez (*), Yolanda Cruz Gómez, Carlos Alfonso Sabatier, Caridad Rojas Mosquera

Hospital Militar Docente Dr. Mario Muñoz Monroy, Matanzas (10)
Ivis Umpierre García

Hospital General Universitario Dr. Gustavo Aldereguía Lima, Cienfuegos (9)
Orelvis Martínez Martínez, Alfredo Basilio Quiñones Ceballos, Mailin Gutiérrez Rodríguez

Hospital Provincial Universitario Arnaldo Milián Castro, Villa Clara (8)
Antonio Moret Pérez, Merlyn Arse Núñez, Candelaria Amada Ramos Collado, Lutgarda Pérez de Alejo, Galia Averhoff, Carmen Garrido Arce

Hospital Provincial Docente Clínico Quirúrgico Manuel Ascunce Domenech, Camagüey (8)
Luis Lauriano Soler Porro, Verónica García Yllán, Alexander Padrón Echevarría

Hospital Clínico Quirúrgico Dr. Salvador Allende, La Habana (7)
Bárbara Castellanos Fernández

Hospital Clínico Quirúrgico Interprovincial Saturnino Lora, Santiago de Cuba (6)
Sergio del Valle Díaz, Lissette Espinoza Martín, Teresa Gómez Álvarez, Reina Aurora Coello Salazar, María Luisa Correoso

Hospital Clínico Quirúrgico Docente José Ramón López Tabrane, Matanzas (6)
José Manuel González González, Aleida Corso González, Manuel Ríos Serrano

Hospital Clínico Quirúrgico Hermanos Ameijeiras, La Habana (5)
Pedro Evelio Velbes Marquetti, Carlos Domínguez Alvarez, Tania Isabel Carballo Treto, Yosagne Soto Matos

Hospital General Provincial Dr. Ernesto Che Guevara, Las Tunas (4)
Nelson Hidalgo Gutiérrez, Idania Molina Raad

Hospital Clínico Quirúrgico Miguel Enríquez, La Habana (3)
Nancy Jiménez Sánchez, María M Rubio Pampin, Antonio Suárez Núñez, Mirna García Meneses

Hospital Clínico Quirúrgico Provincial Celia Sánchez Manduley, Granma (3)
Carmen Villadúniga Reyes, Aida Naranjo Torres, Arturo Ramírez Céspedes, Manuel Aguilera Hernández, Dianelis Alarcón Ferrera

Hospital General Docente Dr. Enrique Cabrera Cossío, La Habana (2)
Gladys Barreto Cruz, Diana Torres López, Ramón Portales Pérez, Gerardo Laza Díaz

Hospital Docente Celestino Hernández Robau, Villa Clara (1)
Pedro Juan Vázquez González

Hospital General Docente Vladimir Ilich Lenin, Holguín (1)
Agustín Mulet Rodríguez

Hospital General Provincial Universitario Camilo Cienfuegos Gorriarán, Sancti Spíritus (1)
Lourdes Pérez Camejo

Hospital Universitario Clínico Quirúrgico Dr. Agostinho Neto, Guantánamo (1)
Leopoldo Lage Canedo

Analysis of samples in laboratories of Molecular Biology

Centro de Inmunoensayo, La Habana
Yaime González González, Anny Armas Cayarga, Iria García de la Rosa, Idania González Pérez, Yenitse Perea Hernández

Instituto de Medicina Tropical Pedro Kourí, La Habana
Licel de los Angeles Rodríguez Lay, Susel Sariego Frómeta, Marité Bello Corredor, Madelin Blanco de Armas

Centro Nacional Coordinador de Ensayos Clínicos, La Habana
María Amparo Pascual López, Grisel Soto Arguelles, Mayté Amoroto Roig (*), Yuliet Bermúdez Hernández, Miriela Fernández Cervera, Odalys González Díaz, Débora Monterrey Cao, Yordanka Martínez Armengol, Clara Mercedes Ballagas Flores

Facultad de Medicina, Pinar del Río
Karina Miranda Hernández, Yoryana Ramírez Sánchez, Ernesto Álvarez Fontanet

Facultad de Medicina, Matanzas
Sandra Naranjo Rodríguez

Facultad de Medicina, Cienfuegos
Ana Ramos Cedeño, Leslie Perez Ruiz

Instituto Superior de Ciencias Médicas, Villa Clara
Migdacelys Arbolaez Estrada, Migdalia Rodríguez Rivas

Facultad de Medicina, Sancti Spíritus
Héctor Ruiz Calabuch

Instituto Superior de Ciencias Médicas, Camagüey
Ileana Pérez Chong, Ana R Valls Hung

Facultad de Medicina, Las Tunas
Norma Francisca Montes de Oca Escobar, Damaris Peña Cuervo

Universidad de Ciencias Médicas, Holguín
Zaimar Rodríguez Feria, Dulce Mariño Cruz

Facultad de Medicina, Granma
Olga Roselló Salcedo

Instituto Superior de Ciencias Médicas, Santiago de Cuba
Doris Perdomo Leyva, Yaimarelis Saumell Nápoles

Facultad de Medicina, Guantánamo
Lorenzo Dorado de la Haye

Data management, statistical design and analyses

Centro de Investigaciones Biológicas, La Habana
Elizeth García Iglesias (*), Leovaldo Alvarez Falcón, Laura Pereda Lamela, Yunia Delgado Castillo, Grettel Melo Suárez, Karina Mireya Mazaira Behar

Note: The Centro de Investigaciones Biológicas, CIB, is a unit of the Centro de Ingeniería y Biotecnología of Havana.

Trial protocol design, monitoring and results analyses

Centro de Investigaciones Biológicas, La Habana
Hugo Nodarse Cuní (*), Odalys Caridad Lazo Diago, Pedro Antonio López Saura (*), Cimara Hortensia Bermúdez Badell, Ketty Cruz Chirino