Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: The BENznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT)

Jose Antonio Marin-Neto, MD,[a] Anis Rassi, Jr, MD,[b] Carlos A. Morillo, MD,[c] Alvaro Avezum, MD,[d] Stuart J. Connolly, MD,[c] Sergio Sosa-Estani, MD,[e] Fernando Rosas, MD,[f] and Salim Yusuf, MD[c] on behalf of BENEFIT Investigators ***Sao Paulo and Goiania, Brazil; Hamilton, Ontario, Canada; Buenos Aires, Argentina; Bogota, Colombia***

Background Benznidazole is effective for treating acute and chronic (recently acquired) *Trypanosoma cruzi* infection (Chagas' disease). Recent data indicate that parasite persistence plays a pivotal role in the pathogenesis of chronic Chagas' cardiomyopathy. However, the efficacy of trypanocidal therapy in preventing clinical complications in patients with preexisting cardiac disease is unknown.

Study Design BENEFIT is a multicenter, randomized, double-blind, placebo-controlled clinical trial of 3,000 patients with Chagas' cardiomyopathy in Latin America. Patients are randomized to receive benznidazole (5 mg/kg per day) or matched placebo, for 60 days. The primary outcome is the composite of death; resuscitated cardiac arrest; sustained ventricular tachycardia; insertion of pacemaker or cardiac defibrillator; cardiac transplantation; and development of new heart failure, stroke, or systemic or pulmonary thromboembolic events. The average follow-up time will be 5 years, and the trial has a 90% power to detect a 25% relative risk reduction. The BENEFIT program also comprises a substudy evaluating the effects of benznidazole on parasite clearance and an echo substudy exploring the impact of etiologic treatment on left ventricular function. Recruitment started in November 2004, and >1,000 patients have been enrolled in 35 centers from Argentina, Brazil, and Colombia to date.

Conclusion This is the largest trial yet conducted in Chagas' disease. BENEFIT will clarify the role of trypanocidal therapy in preventing cardiac disease progression and death. (Am Heart J 2008;156:37-43.)

From the [a]Medical School of Ribeirao Preto, University of Sao Paulo, Ribeirão Preto, Sao Paulo, Brazil, [b]Anis Rassi Hospital, Goiania, Brazil, [c]McMaster University, Population Health Research Institute HGH-McMaster Clinic, East Hamilton, Ontario, Canada, [d]Instituto Dante Pazzanese de Cardiologia, Sao Paulo, Brazil, [e]Servicio de Epidemiología del Centro Nacional Diagnóstico e Investigación de Endemo-epidemias, Administración Nacional de Laboratorios e Institutos de Salud "Dr. Carlos G. Malbrán", Buenos Aires, Argentina, and [f]Clinica A. Saio, Bogota, Colombia.

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Reprint requests: Jose Antonio Marin-Neto, MD, PhD, FACC, is to be contacted at South America, Medical School of Ribeirao Preto, University of Sao Paulo, 14048-900 – Ribeirão Preto, SP – Brazil. Carlos A. Morillo, MD, FRCPC, FACC, North America, McMaster University - Population Health Research Institute, HGH-McMaster Clinic 5th Floor Room 501 237 Barton St. East Hamilton, ON, Canada L8L 2X2.

E-mails: marin_neto@yahoo.com, morillo@hhsc.ca

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Chagas' disease (CD) represents the third largest parasitic disease burden globally after malaria and schistosomiasis.[1] Chronic Chagas' cardiomyopathy (CCC) is the most common form of nonischemic cardiomyopathy worldwide, and one of the leading causes of morbidity and death in Latin America.[2] It is estimated that 10 to 12 million people are infected, and 25% to 30% of these will develop cardiomyopathy, with more 15,000 annual deaths attributed directly to this etiology[3,4] Approximately 220,000 new cases yearly, and 667,000 disability-adjusted life years are lost annually.[5]

Chagas' disease is caused by *Trypanosoma cruzi*, transmitted to humans by triatominae insects, blood transfusion, oral contamination, or congenitally.[6] Chagas' disease has 2 clinical phases: acute and chronic. Acute infection may manifest by a self-limited febrile illness that lasts 4 to 8 weeks but is often unrecognized. Mortality in this phase is <5%, and deaths are due to myocarditis or meningoencephalitis.[7] The chronic phase appears after a long latent period (indeterminate

form), which lasts throughout life in about two thirds of patients. Individuals in the indeterminate form have no symptoms or electrocardiographic (ECG) or radiologic evidence of involvement of the heart or gastrointestinal tract.[8] The remaining one third of chronically infected individuals develop cardiac or digestive complications 10 to 30 years after the initial infection.

Cardiac involvement is the most frequent and serious consequence of chronic CD and typically produces atrial and ventricular arrhythmias, conduction disturbances, wall motion abnormalities, cardiac failure, pulmonary and systemic thromboembolism, and sudden death.[2,6] Annual mortality for outpatients has been estimated to be about 4%.[9] Sudden death accounts for 55% to 65% of the deaths; heart failure, 25% to 30%; and thromboembolic phenomena, for the remaining 10% to 15%.[10]

Rationale for trypanocidal treatment in CCC

The pathogenesis of CD is unclear. Although *T cruzi* causes acute CD and the benefits of trypanocidal treatment (TT) in that stage are undisputable, the role of the parasite and the impact of treatment in the chronic phase are more controversial.[11,12] Several studies have implicated autoimmune phenomena as the principal mechanism leading to late cardiac injury.[10-15] This hypothesis is based on the apparent absence of parasites in the cardiac inflammatory lesions and the presence of anti–self-immune responses in CCC patients, caused either by autoantibodies or autoreactive T cells, derived by molecular mimicry between parasite and host antigens.[16] This hypothesis suggests that etiologic treatment would be of little benefit.[17]

The demonstration of *T cruzi* antigens in inflamed myocardium by more sensitive methods, such as immunohistochemistry and polymerase chain reaction (PCR), suggests that vestiges of parasites are necessary to trigger the inflammatory process.[18-20] Although *T cruzi* genetic material has not been detected in the heart from seropositive autopsied patients dying without evidence of cardiac involvement, it was consistently found in the heart and esophageal specimens from patients with these organs affected.[18,21] Furthermore, TT attenuates the pathologic consequences in experimental models reducing parasite burden.[22,23] Conversely, immunosuppressive treatments aggravate inflammatory response in experimental models and in humans.[24-26]

In summary, current knowledge suggests that the pathogenesis of CCC is dependent on a low-grade, persistent parasite presence, coupled with the participation of antiparasite and/or anti–self-immune responses.[11,12,27] Thus, elimination of *T cruzi* may avert its long-term consequences.

Efficacy of benznidazole in CD

Only 2 nitroheterocyclic drugs, nifurtimox and benznidazole, introduced in the mid 1960s and early 1970s, respectively, demonstrated significant trypanocidal activity in the acute and recent chronic phases of intection.[28,29]

Benznidazole (*N*-benzil-2-nitro-1-imidazole-acetamide) has direct action against both the circulating (trypomastigote) and tissular (amastygote) forms of *T cruzi*. Efficacy varies according to phase of CD, dose and duration of treatment, age, length of follow-up after therapy, and tests used to assess parasite clearance. Acutely, cure rates of 60% to 80% have been reported.[7] In the early chronic phase of *T cruzi* infection, 2 randomized placebo controlled trials tested the efficacy of benznidazole in schoolchildren from Brazil[30] and Argentina.[31] Negative seroconversion was achieved in 58% of the benznidazole-treated patients by the end of 3-year follow-up in the Brazilian trial and in 62% of the Argentinean children by the end of 4-year follow-up. A systematic review of trypanocidal drugs for chronic asymptomatic *T cruzi* infection analyzed the results of 5 randomized clinical trials involving 756 participants and concluded that nitroimidazole derivatives, especially benznidazole, improved parasite-related outcomes in both children and adults.[32] Overall, benznidazole reduced the proportion of positive xenodiagnosis by 81% and led to an 11-fold increase in the rate of negative seroconversion.[32] However, whether the reduction in parasite load translates into improved clinical outcomes was not assessed.

The role of antiparasitic treatment in the late chronic phase of CD remains unclear.[33-39] Some nonrandomized studies suggested that treatment was associated with negativization of serologic tests and prevention of clinical and ECG evolution,[33,37-39] but others yielded inconclusive results.[35,36] Moreover, 2 independent analyses of pooled data from observational studies in the late stage of CD showed that there is insufficient evidence to support the use of trypanocidal drugs for chronic *T cruzi* infection.[40,41] A well-designed clinical trial is needed to determine whether TT can favorably affect the natural history of CCC.

We describe the design of the BENznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT) program, which consists of 2 phases: the BENEFIT pilot study addressing safety and tolerability of benznidazole and its efficacy for reducing parasite burden in patients with CCC and the BENEFIT full-scale trial will assess the effect of TT on the clinical progression of CCC.

Study design

BENEFIT pilot study

The primary objective of the BENEFIT double-blind randomized pilot study is to determine the efficacy of

Table I. Inclusion criteria

≥1 of the following (A through E):
A. Abnormal electrocardiogram (at least 2 of the following):
1. Right bundle-branch block
2. Left bundle-branch block
3. Left anterior fascicular block
4. Left posterior fascicular block
5. Ventricular premature beats
6. First degree AV block >220 milliseconds, in absence of drugs slowing AV conduction
7. Mobitz type I AV block, in absence of drugs slowing AV conduction
8. Sinus bradycardia <50 beat/min or sinus pauses >3.0 s, in absence of sinus node blocking drugs
9. Primary ST-T changes
10. Abnormal Q waves
11. Low voltage QRS
12. Atrial fibrillation
B. Abnormal ECG (one of the following):
1. Mobitz type II, advanced or third degree AV block
2. Cardiac pacemaker or implanted automatic defibrillator
C. Increased cardiothoracic ratio (>.50)
D. Complex ventricular arrhythmias (multiform >10/h, couplets or NSVT) on 24-h ECG monitoring
E. Evidence of regional wall motion abnormality or reduced (<50%) global LV systolic function (2D Echo, RNA, contrast ventriculography) or increased LV end-diastolic diameter (>55 mm) on 2D Echo

AV, Atrioventricular; *NSVT*, non-sustained ventricular tachycardia.

Table II. Exclusion criteria

a. New York Heart Association class IV or decompensated heart failure:
b. Evidence of concomitant coronary artery disease or other etiology of dilated cardiomyopathy;
c. Previous treatment with trypanocidal agents or an accepted indication for antiparasitic therapy (eg, reactivation of Chagas' infection due to immunosuppression by several diseases or treatment with steroids);
d. Inability to comply with follow-up;
e. History of severe alcohol abuse, or any other drug addition within past 2 years;
f. Known chronic renal failure (serum creatinine >1.5 mg/dL) or hepatic insufficiency (AST/ALT >3× normal);
g. Pregnancy or breast feeding;
h. Megaesophagus with severe swallowing impairment;
i. Other diseases significantly curtailing life expectancy

AST, Aspartate amino-transferase; *ALT*, alanine amino-transferase.

benznidazole given for 60 days in reducing parasite burden and its safety in 600 patients with CCC.

The coprimary outcomes of the pilot study are: (1) negativization of *T cruzi* detection by PCR[42] and (2) reduction in the mean parasite load as assessed by the concentration of *T cruzi* per milliliter of blood by real-time polymerase chain reaction (PCR).[43] Polymerase chain reaction will be performed at randomization, at end of therapy (60 days), and at 2 years of follow-up.

BENEFIT full-scale trial

The primary objective of the BENEFIT full-scale trial is to evaluate whether TT reduces mortality and major cardiovascular clinical outcomes: composite of death, resuscitated cardiac arrest, pacemaker or cardiac defibrillator insertion, sustained ventricular tachycardia, cardiac transplantation, development of new heart failure, stroke or systemic or pulmonary thromboembolic event.

The secondary objectives are to determine if etiologic treatment reverses or halts left ventricular (LV) dysfunction, influences the development of ECG alterations, or reduces symptoms and parasite burden.

Patient eligibility

Chagasic patients aged ≥18 years and ≤75 years, are eligible if, in addition to having any combination of at least 2 positive serologic tests for CD (indirect immunofluorescence, indirect hemagglutination, or ELISA), have evidence of cardiomyopathy based on the criteria outlined in Table I.

Exclusion criteria are summarized in Table II.

Patients living in housing conditions that predispose to *T cruzi* reinfection will not be excluded; general measures are implemented to assure elimination of vectorial transmission in areas where such patients are enrolled.[3] Sensitivity analysis will be performed to assess consistency of results.

Randomization and follow-up

Patients are randomly assigned to placebo or benznidazole 5 mg/kg per day during 60 days. Randomization will be 1:1 with stratification according to centre using a random-block system. Scheduled follow-up visits occur at 11, 21, and 2 months after initiation of treatment and will be followed up at 6 months and then annually until a minimum of 4 and a maximum of 6 years are reached.

Sample size and data analysis

Six patients with positive detection of parasite by PCR at baseline will be recruited in the pilot trial. Spontaneous negativization rate is expected in 20% to 30% of patients receiving placebo.[43-45] The sample size calculations are shown for 2 possible treatment effects, 50% and 100% relative increase in negativization for the 2 expected rates of spontaneous negativization (20% and 30%). With 0.04 of the 2-sided α devoted to this analysis, there is excellent power to detect doubling of negativization (increase of 100%) and reasonable power to detect 50% increase in negativization within the range of spontaneous negativization expected in the control group (Table III).

The pilot study is well powered to detect a reduction in mean parasite load between the 2 groups after 2 years[43,44] and detect a relative reduction of 25% in parasite load (or an absolute reduction of 6.5) with benznidazole (Table III).

Three thousand patients are needed for the full-scale trial (1,500 per group) and is the minimum required to detect a 26% relative risk reduction (RRR) in the risk of the

Table III. Pilot study sample size

Statistical power for the 2 study coprimaries with 80% of the study power (2-α = .04) dedicated to comparison of the rates of negativization and 80% of power (2-α = .01) dedicated to comparison of group mean parasite burdens

Difference in PCR negativization rates with total of 600 patients

PCR negativization rate in control patients	PCR negativization rate in treated patients	Statistical power (2-α = .04)
20%	30% (50% relative increase)	75%
20%	40% (100% relative increase)	>99%
30%	45% (50% relative increase)	95%
30%	60% (100% relative increase)	95%

Reduction in group mean PCR detected parasite burden for 600 patients

Control parasite burden: mean = 26, SD = 20

Relative reduction	Absolute decrease	Statistical power (2-α = .01)
20%	5.2	72%
25%	6.5	91%
33%	8.6	>99%

composite end point (death, resuscitated cardiac arrest, cardiac transplantation, development of new heart failure, life-threatening nonfatal arrhythmias, thromboembolism, and need for pacemaker or cardioverter defibrillator implantation) with 90% power, assuming a yearly event rate of 8.0% in the control group and 4 to 6 years of follow-up (at 2-sided α of .05). The reported rates of noncompliance with benznidazole are around 17%, and we expect a loss to follow-up of 3% (Table IV).

Data analysis

All analyses of primary and secondary outcomes will be performed according to the intention-to-treat principle. The pilot study will also be useful to determine the actual event rate in this population.

Pilot study. Comparison was done between rates of negativization of parasite detection by PCR (first coprimary outcome) between the benznidazole and placebo groups. Logistic regression will be performed for the rate of negativization at 2 years of follow-up.

The second coprimary outcome is the difference in parasite load between the 2 treatment groups also at 2 years of follow-up. This will be tested using analysis of variance techniques at a 2-sided α of .01.

Full-scale study. The primary analysis of the full-scale BENEFIT trial will be done by comparing time to the first occurrence of any element of the primary composite outcome. Patients lost to follow-up will be censored at the last time of observation. Cox proportional hazards model will be used to investigate the influence of important confounders and prognostic factors. A sensitivity analysis will be performed in patients with prior

Table IV. Sample size calculations for the full-scale study

% Event year primary outcome	Detectable RRR (n = 2000)	Detectable RRR (n = 2500)	Detectable RRR (n = 3000)
6	34.9	31.3	28.8
7	32.7	29.6	27.0
8	30.6	27.6	25.6
9	29.0	26.5	24.1
10	28.0	25.4	23.0

sustained ventricular tachycardia, previous insertion of pacemaker or defibrillator, thromboembolic phenomena or heart failure hospitalization. A prespecified subgroup analysis based on the severity of CCC at admission will also be performed. Severity of CCC will be graded according to the recently developed Rassi score.[9]

Echocardiographic substudy. Patients will have a baseline echocardiogram before treatment allocation and another one at the end of follow-up. All analyses will be blinded and carried centralized at a core laboratory. New wall motion abnormalities and deterioration of global LV function will be determined using standard methods.

Study organization

The BENEFIT study network includes 8 countries and 60 centers, with the Latin American coordinating center located at the Dante Pazzanese Institute in Sao Paulo, Brazil, and the overall coordinating center at the Population Health Research Institute (PHRI), Hamilton Health Sciences, and McMaster University, East Hamilton, Ontario, Canada.

All forms are saved at the regional coordinating center in Sao Paulo and transferred on a weekly basis to the PHRI Project Office. Queries and quality control reports are immediately generated by the Web-based database and sent to investigators. The overall responsibility for the conduct of the trial lies with the Steering Committee (see Appendix A). An operations committee, with representatives from the PHRI Project Office, the regional coordinating centers, and the national coordinators, meets regularly.

Central event adjudication and centralized analyses

A central adjudicator at the project office, blinded to treatment assignment and using essential supportive documentation to confirm the diagnosis, adjudicate study outcomes.

Detection of parasite and measures of parasite burden by PCR will be done at a core laboratory for each involved country.

Serious adverse events and unblinding

All serious adverse events, including primary, secondary, and other study outcomes in the randomization and

follow-up periods are reported to the project office. All serious adverse events are tabulated and reviewed periodically by the independent data and safety monitoring board (DSMB). Central emergency unblinding is available when necessary.

Ethics and patient confidentiality

The protocol has been approved by international regulatory agencies, by all participating institutions, and by the national ethics review committees of the countries already involved in the program. All patients provide written informed consent.

Interim analysis and data monitoring

The DSMB will periodically monitor the trial for safety and efficacy. No formal boundaries are proposed for safety, but clear, consistent, and persistent evidence of net harm that overwhelms any benefit should be apparent.

The-full scale trial will be an event-guided trial, and recommendation to stop the trial will be based on the pattern of treatment effect across all end points, as well as the benefit/risk ratio. Two interim analyses to assess futility are scheduled at approximately one half and three fourths of the total of anticipated events. The trial may be stopped for efficacy if a reduction in events by a 4-fold SD or a 3-SD excess occurs in the first half of the trial or if a reduction in events by 3-SD or a 2-SD excess is detected in the second half of the trial. If the upper limit of the 95% CI for the conditional power for the primary outcome falls <15%, then, all other things being equal, the DSMB may recommend early termination.

Trial progress

Recruitment was initiated in November of 2004 and currently enlists 35 centers that enrolled over 1,000 patients in Brazil, Argentina, and Colombia as of January 2008. Center activation is ongoing and Venezuela, Bolivia, Peru, and Guatemala will join the BENEFIT program for the full-scale trial. Overall, 96% of patients have received over 75% of the assigned treatment during the 60-day period. The overall cumulative rate of drug interruptions is 14.5%, with 6.6% of these patients restarting the assigned treatment.

Discussion

Conventional parasitological methods (xenodiagnosis and hemoculture) for establishing cure rates in the chronic phase have marked inherent limitations.[44] In contrast, a higher sensitivity in parasite detection in the chronic stage has been reported when PCR methods were used,[42] and variable negativization rates are described after TT when *T cruzi* detection was based on this technique.[43-45] These findings raise the possibility that a more sensitive method for parasite detection, such as PCR, could make it a suitable tool for the follow-up assessment of chemotherapy in patients with CCC. Furthermore, even if persistence of PCR detection of *T cruzi* is considered a therapeutic failure, assessment of parasite load by quantitative real-time PCR could still be correlated with the impact of TT on the evolution of disease.

Apart from clear limitations in the objective markers of *T cruzi* eradication and the lack of appropriately designed and statistically powered randomized clinical trials, establishing the role of etiologic treatment in patients with CCC is hindered for 2 other reasons: incomplete knowledge about the natural history and uncertainty regarding the pathogenesis of the disease.

The BENEFIT programs will address important questions which remain unresolved. First, the safety and efficacy of benznidazole in patients with CCC will be evaluated in the pilot study with modern methods for assessment of parasitological cure (real-time PCR). Second, it will assess if a significant reduction in parasitic load may serve as an alternative end point of TT, so that complete clearance of parasites may not be essential to achieve a delay in the progression of the disease. Third, BENEFIT will determine whether TT leads to a reduction in hard clinical outcomes; in addition, extending previous studies in patients with a large spectrum of clinical manifestations of cardiac involvement,[33,39] BENEFIT will test if etiologic therapy can delay the progression of CCC. Obtaining a definitive answer to this last question will indeed have a major impact on the management of this neglected disease. In addition, BENEFIT is the first multinational clinical trial conducted in CD and will create a network of investigators that will hopefully continue to address important issues in these patients. Finally, the BENEFIT program will determine a number of outcomes related with parasitological and clinical characteristics in several countries. Differences in clinical manifestations between southern cone and more northern strains of *T cruzi* have been suggested but are not clearly established. The BENEFIT program provides a unique opportunity for better understanding the clinical progression of CCC and will provide conclusive information on the role of etiologic treatment in this phase of the disease. This need is reflected by the fact that, mostly on arbitrary basis, current guidelines in Latin American countries, extensively reviewed on a recent publication, recommend etiologic treatment aiming at slowing the development and progression of cardiomyopathy for patients <50 years, but not for older subjects.[46]

Collaboration of Canadian investigators with those in Latin America is also structured to develop capacity and expertise to tackle important questions through the conduct of randomized clinical trials, which eventually can be used to address important questions of public health in this region. Improving global health requires such collaborations and empowering clinician scientists in developing countries to approach important

questions of local relevance. The BENEFIT trial was deliberately structured to develop capacity to coordinate trials in Latin America, and this may likely be its most important contribution, as it can form a foundation on which several future trials and epidemiological questions could be addressed.

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Appendix A

Steering committee

S Yusuf (Chair), JA Marin-Neto (Cochair, Co–Principal Investigator), CA Morillo (Co–Principal Investigator), A Rassi, A Rassi Jr, A Avezum, SJ Connolly, S Sosa-Estani, F Rosas, C Britto, J Lazdins, B Ramos

Operations committee

C Morillo (Chair), A Avezum (Cochair), S Yusuf, JA Marin-Neto, R Oliveira, L Dyal, H Penna-Guimarães, AC Mattos, A Rassi Jr, C Britto, S Sosa-Estani, E Segura, F Rosas, F Guhl, J Lazdins, BC Maciel

Data and safety monitoring board

P Sleight (Chair), H Acquatella, J Lazzari, D Sackett, R Robins

Coordinating centers

Population Health Research Institute, McMaster University, Hamilton, Canada
(C Morillo, S Yusuf, B Ramos, R Oliveira),
Instituto Dante Pazzanese, São Paulo, Brazil (A Avezum, H Penna-Guimaraes, AC Mattos, JR Zappiello-Mendes, Juarez Braga)
Medical School of Ribeirao Preto, University of São Paulo, Brazil (JA Marin-Neto, BC Maciel, Tonani)

Principal investigators by country

Argentina: S Sosa-Estani (National Coordinator [NC]), TA Orduna, FA Silva-Nieto, MP Bernachea, R Carrizo Pae, R Manzur, CA Cuneo, G Perez-Pardos, J Beloscar, E Oshiro, MC Bangher, L Gomes, VI Volberg, M Mallagray.

Brazil: A Rassi Jr (NC), A Luquetti, A Schmidt, AA Fragata Filho, L Nigro-Maia, AML Lorga, AS Souza, RC Pedrosa, ED Gontijo, WA Oliveira Jr, R Aras, GS Feitosa, A Prata, TL Silva Jr, AA Steffens, CLP Cunha, LFA Oliveira, AC Carvalho, ACA Souza, JFK Saraiva, C Mady, EA Bocchi, G Reis, AA Fagundes.

Colombia: F Rosas (NC), S Navarrete, R Quiroz, R Onate

Bolivia: E Villena (NC)

Guatemala: B Arana (NC)

Peru: A Laguna (NC)

Venezuela: R Espinosa (NC)