

# Cuban Research in Current International Journals

The following selection—alphabetical by title—reflects Cuban publishing in international medical and population health journals over the last quarter on an array of topics. Links to these journal articles may be found at [www.medicc.org/mediccreview](http://www.medicc.org/mediccreview).

**A clinical exploratory study with itolizumab, an anti-CD6 monoclonal antibody, in patients with rheumatoid arthritis.** Rodríguez PC, Torres-Moya R, Reyes G, Molinero C, Prada D, Lopez AM, et al. *Results Immunol.* 2012 Nov 20. [Epub ahead of print]

T cells are involved in the pathogenesis of rheumatoid arthritis (RA). CD6 is a co-stimulatory molecule, predominantly expressed on lymphocytes, that has been linked to autoreactive responses. The purpose of this study was to evaluate the safety, immunogenicity and preliminary efficacy of itolizumab, a humanized anti-CD6 monoclonal antibody, in patients with active rheumatoid arthritis. Fifteen patients were enrolled in a phase I, open-label, dose-finding study. Five cohorts of patients received a weekly antibody monotherapy with a dose-range from 0.1 to 0.8 mg/kg. Itolizumab showed a good safety profile, with no severe or serious adverse events reported so far. No signs or symptoms associated with immunosuppression were observed in the study. Objective clinical responses were achieved in more than 80% of patients after treatment completion, and these responses tend to be sustained afterwards. This clinical study constitutes the first evidence of the safety and positive clinical effect of a monotherapy using an anti-CD6 antibody in patients with rheumatoid arthritis.

**Anti-Epidermal Growth Factor/Epidermal Growth Factor Receptor Therapeutic Anti-cancer Drugs and the Wound Healing Process.** Casacó A, Fuente D, Ledón N, Fernández A, Crombet T. *J Cancer Sci Ther.* 2012 Oct;4(10):324–9.

Cutaneous wound healing is a complex process involving blood clotting, inflammation, tissue formation, and tissue remodelling. Many experimental and clinical studies have demonstrated varied, but in most cases beneficial, effects of exogenous growth factors on the healing process. The use of targeted anti-cancer agents is increasing. It is common to utilize a multi-modal treatment approach towards solid tumors, often including surgical resection, and it has become apparent that some targeted agents can impair wound healing or cause increasing risk of peri-operative complications. There are limited data regarding the wound healing process of anti-cancer target drugs blocking the EGF/EGFR system. The aim of this paper is to review and to comment the effects of anti-EGF/EGFR drugs on the skin wound healing process after programmed or emergency surgical procedures. A review of the current literature, including our own results, was undertaken. We included the monoclonal anti-

bodies cetuximab, panitumumab, nimotuzumab; the small tyrosine kinase molecules erlotinib and gefitinib; and the EGF-based cancer vaccine; CIMAvax and the EGFR-based cancer vaccine; HER-1 vaccine. Apparently, there are no deleterious effects of the anti-EGF/EGFR drugs in the wound healing post-operative process. Taking into account that treatment with anti-EGF/EGFR drugs inhibits tumor cell proliferation, and the lack of deleterious effects of these EGF/EGFR specific inhibitors in the wound healing post-operative process; we suggest that these kinds of drugs could be maintained and their effects tested, with very special surveillance during the post-surgical period.

**Balancing the Risks and Benefits of the Use of Over-the-Counter Pain Medications in Children.** Bárzaga Arencibia Z, Choonara I. *Drug Saf.* 2012 Dec 1;35(12):1119–25.

Paracetamol (acetaminophen) and ibuprofen are the most frequently purchased over-the-counter (OTC) medicines for children. Parents purchase these medicines for the treatment of fever and pain. In some countries other NSAIDs such as aspirin (acetylsalicylic acid) and dipyron are available. We aimed to perform a narrative review of the efficacy and toxicity of OTC analgesic medicines for children in order to give guidance to health professionals and parents regarding the treatment of pain in a child. Neither aspirin nor dipyron are recommended for OTC use because of the association with Reye's syndrome for the former and the risk of agranulocytosis for the latter. Both paracetamol and ibuprofen are effective for the treatment of mild pain in children. Adverse effects with both medicines are infrequent. Ibuprofen is an NSAID and therefore there is a greater risk of gastrointestinal adverse effects and hypersensitivity. Aspirin and dipyron should be avoided. Paracetamol is the drug of first choice for mild pain in children because of its favourable safety profile. For the treatment of significant musculoskeletal pain, ibuprofen is the drug of first choice.

**Clinical and microbiological characterization of pneumonia in mechanically ventilated patients.** Medell M, Hart M, Marrero O, Espinosa F, Montes de Oca Z, Valdés R. *Braz J Infect Dis.* 2012 Sep–Oct;16(5):442–7.

**Objective** To characterize mechanical ventilation-associated pneumonia (MVAP). **Method** This is an observational descriptive study to characterize MVAP in 61 ventilated patients admitted in the intensive care units of the Hermanos Ameijeiras hospital during 2011. This

study also aimed to isolate the bacteria causing MVAP and characterize their resistance to antibiotics. **Result** 51 (83.60%) patients presented pulmonary infiltrates and 35 (50.81%) presented a clinical score  $\geq 6$  according to the Clinical Pulmonary Infection Score. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were the most frequently isolated microorganisms from patients with MVAP. Both microorganisms showed a high resistance to antibiotics. Carbapenems were the most frequent used antimicrobial therapeutic agents; elective antibiotic combinations were directed against both bacterial wall structure and nucleic acid synthesis. **Conclusion** Patients with MVAP identified during the studied period showed similar frequency to those reported in medical literature. Thus, this study corroborated that this is still a relevant medical problem in this hospital. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were the most frequently isolated microorganisms from patients with MVAP. Antimicrobial treatment, empirical or not, are still the main risk factors for the development of multidrug-resistant strains of bacteria. The rate of resistance to antibiotics of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* strains isolated from patients with MVAP was higher than those isolated from infected patients without MVAP. Tigecycline and colistin were the only antibiotics fully effective against *Acinetobacter baumannii* strains isolated in 2011 from patients with MVAP; against *Pseudomonas aeruginosa* strains, only colistin was fully effective.

**Conventional polymerase chain reaction for the diagnosis of neurotoxoplasmosis: comparison of three sets of primers for the B1 gene using CSF samples.** Alfonso Y, Fraga J, Cox R, Jiménez N, Capó V, Pomier O, et al. *Diagnos Microbiol Infect Dis.* 2012 Nov;74(3). [Epub ahead of print]

Polymerase chain reaction (PCR) has made a significant improvement in the diagnosis of toxoplasmic encephalitis (TE). Nevertheless, a wide variety of targets and primers has been used in different assays, and few comparative studies had been carried out. The aim of the present study was to compare the efficiency of 3 conventional PCR methods by using 3 sets of primers targeting the repetitive B1 gene in the diagnosis of TE. Diagnostic sensitivity and specificity of PCR and nested-PCR protocols were assessed for 207 (nested-PCR/T1-T4), 200 (nested-PCR/S1-AS1), and 206 (PCR/B22-B23) cerebrospinal fluid (CSF) samples, including AIDS and HIV-negative patients. The diagnostic sensitivity of PCR and nested-PCR assays was 50.85%, 68.97%, and 72.41% for

T1-T4, S1-AS1, and B22-B23, respectively. The diagnostic specificity was high for all the assays showing values between 95% and 97%. In general, the best results were obtained for the B22-B23 set of primers, suggesting their usefulness compared with 2 nested-PCR protocols and showing that this simple and rapid strategy may be the preferred one for the diagnosis of TE in AIDS patients.

**Effect of vaccination with N-glycolyl GM3/VSSP vaccine by subcutaneous injection in patients with advanced cutaneous melanoma.** Osorio M, Gracia E, Reigosa E, Hernández J, de la Torre A, Sáurez G, et al. *Cancer Management Res.* 2012 Oct;4:347–50.

NeuGc-containing gangliosides have been described in melanoma cells and are an attractive target for cancer immunotherapy because they are minimally or not expressed in normal human tissues. Melanoma patients treated with a vaccine based on N-glycolyl gangliosides have shown benefit in progression free survival and overall survival. We conducted a multicenter Phase I/II clinical trial in patients with metastatic cutaneous melanoma treated with the N-glycolyl GM3/very-small-size proteoliposomes vaccine by the subcutaneous route. Selecting the optimal biological dose of the vaccine was the principal objective based on immunogenicity, efficacy, and safety results. Six dose levels were studied and the treatment schedule consisted of five doses administered every 2 weeks and then monthly until 15 doses had been given. Dose levels evaluated were 150, 300, 600, 900, 1200, and 1500 µg with five patients included in each dose level except the 900 µg dose (n = 10). Immunogenicity was determined by antibody titers generated in patients after vaccination. Antitumor effect was measured by response criteria of evaluation in solid tumors and safety was evaluated by common toxicity criteria of adverse events. The vaccine was safe and immunogenic at all doses levels. The most frequent adverse events related to vaccination were mild to moderate injection site reactions and flu-like symptoms. Vaccination induced specific anti-NeuGcGM3 immunoglobulin M and immunoglobulin G antibody responses in all patients. Disease control (objective response or stable disease) was obtained in 38.46% of patients. Global median overall survival was 20.20 months. Two patients achieved overall survival duration of about 4 and 5 years, respectively. The 900 µg dose resulted in overall survival duration of 19.40 months and was selected as the biological optimal dose.

**Effectiveness of a multidimensional approach for prevention of ventilator-associated pneumonia in adult intensive care units from 14 developing countries of four continents: Findings of the International Nosocomial Infection Control Consortium.** Rosenthal VD, Rodrigues C, Alvarez-Moreno C, Madani N, Mitrev Z, Ye G, et al. *Crit Care Med.* 2012 Dec;40(12):3121–8.

**Objectives** The aim of this study was to analyze the effect of the International Nosocomial Infection Control Consortium's multidimensional approach on the reduction of ventilator-associated pneumonia in patients hospitalized in intensive care units. **Design** A prospective active surveillance before-after study. The study was divided into two phases. During phase 1, the infection control team at each intensive care unit conducted active prospective surveillance of ventilator-associated pneumonia by applying the definitions of the Centers for Disease Control and Prevention National Health Safety Network, and the methodology of International Nosocomial Infection Control Consortium. During phase 2, the multidimensional approach for ventilator-associated pneumonia was implemented at each intensive care unit, in addition to the active surveillance. **Setting** Forty-four adult intensive care units in 38 hospitals, members of the International Nosocomial Infection Control Consortium, from 31 cities of the following 14 developing countries: Argentina, Brazil, China, Colombia, Costa Rica, Cuba, India, Lebanon, Macedonia, Mexico, Morocco, Panama, Peru, and Turkey. **Patients** A total of 55,507 adult patients admitted to 44 intensive care units in 38 hospitals. **Interventions** The International Nosocomial Infection Control Consortium ventilator-associated pneumonia multidimensional approach included the following measures: 1) bundle of infection-control interventions; 2) education; 3) outcome surveillance; 4) process surveillance; 5) feedback of ventilator-associated pneumonia rates; and 6) performance feedback of infection-control practices. **Measurements** The ventilator-associated pneumonia rates obtained in phase 1 were compared with the rates obtained in phase 2. We performed a time-series analysis to analyze the impact of our intervention. **Main Result** During phase 1, we recorded 10,292 mechanical ventilator days, and during phase 2, with the implementation of the multidimensional approach, we recorded 127,374 mechanical ventilator days. The rate of ventilator-associated pneumonia was 22.0 per 1,000 mechanical ventilator days during phase 1, and 17.2 per 1,000 mechanical ventilator days during phase 2. The adjusted model of linear trend shows a 55.83% reduction in the rate of ventilator-associated pneumonia at the end of the study period; that is, the ventilator-associated pneumonia rate was 55.83% lower than it was at the beginning of the study. **Conclusion** The implementation of the International Nosocomial Infection Control Consortium multidimensional approach for ventilator-associated pneumonia was associated with a significant reduction in the ventilator-associated pneumonia rate in the adult intensive care units setting of developing countries.

**Efficient expression systems for cysteine proteases of malaria parasites: Too good to be true?** Sarduy ES, Planes MD. *Bioengineered.* 2012 Sep 27;4(2). [Epub ahead of print]

Papain-like cysteine proteases of malaria parasites are considered important chemotherapeutic tar-

gets or valuable models for the evaluation of drug candidates. Consequently, many of these enzymes have been cloned and expressed in *Escherichia coli* for their biochemical characterization. However, their expression has been problematic, showing low yield and leading to the formation of insoluble aggregates. Given that highly-productive expression systems are required for the high-throughput evaluation of inhibitors, we analyzed the existing expression systems to identify the causes of such apparent issues. We found that significant divergences in codon and nucleotide composition from host genes are the most probable cause of expression failure, and propose several strategies to overcome these limitations. Finally we predict that yeast hosts *Saccharomyces cerevisiae* and *Pichia pastoris* may be better suited than *E. coli* for the efficient expression of plasmidial genes, presumably leading to soluble and active products reproducing structural and functional characteristics of the natural enzymes.

**Experiences of teacher training in economic evaluation for health professionals in Cuba.**

Collazo M, Fernández A, Martínez JR. *Int J Care Pathways.* 2012 Sep;16(3):67–71.

The objective of this study was to conduct a literature review to assess the importance of teacher training on the economic assessment for professionals working in the Cuban National Health Service (NHS). The assessment will be used as a working tool to guide the process of decision-making in the health sector and to increase the efficiency of different actions to be undertaken in the country. We conducted a literature review to search comprehensive information about this topic at the national and international levels with the intention of incorporating this discipline as a component of crucial importance for the pursuit of efficiency in the Cuban health-care system. Teacher training in economic evaluation applied to the field of health is considered an aspect that improves the rational use of different health technologies, which implies an efficient use of resources for the NHS in Cuba. Although the costs of these therapies are becoming more substantial, their assessment in terms of efficiency allows us to identify effective and less costly interventions for the health sector in Cuba by defining the best strategies to support the process of decision-making. We can confirm that the teacher training in economic evaluation of health professionals for the NHS has been a pressing need. The goal is to develop an economic culture that enables the personnel to show an increased efficiency in performing their normal duties and tasks when developing health interventions that are organized and developed in the country.

**Expression of cell proliferation cycle negative regulators in fibroblasts of an ischemic diabetic foot ulcer. A clinical case report.** Berlanga-Acosta J, Mendoza-Mari Y, Martínez MD, Valdés-Pérez C, Ojalvo AG, Armstrong D. *Int Wound J.* 2012 Nov 30. doi: 10.1111/j.1742-481X.2012.12000.x. [Epub ahead of print]

Wound chronification and opportunistic infections stand as major factors leading to lower extremities amputations in diabetes. The molecular mechanisms underlying diabetic's torpid healing have not been elucidated. We present the case of a female diabetic patient that after a plantar abscess surgical drainage, tight glycaemia control and infection clearance; the wound bed evolved to chronification with poor matrix accumulation, scant angiogenesis and no evidence of dermo-epidermal contours contraction. Ulcer fibroblasts yet cultured under 'physiological' conditions exhibited a slow and declining proliferative response. Diabetic fibroblasts cycle arrest occurred earlier than non-diabetic counterparts. This in vitro premature arrest-senescence phenotype appeared related to the transcriptional upregulation of p53 and the proto-oncogene c-myc; with a concomitant expression reduction of the survival and cellular growth promoters *Akt* and mTOR. Importantly, immunocytochemistry of the diabetic ulcer-derived fibroblasts proved nuclear over expression of potent proliferation inhibitors and pro-senescence proteins as p53 phosphorylated on serine-15 and p21<sup>Cip1</sup>. In line with this, cyclin D1 appeared substantially underexpressed in these cells. We postulate that the downregulation of the *Akt*/mTOR/cyclin D1 axis by the proximal activation of p53 and p21 due to stressor factors, impose an arrest/pro-senescence programme that translated in a torpid and slow healing process.

**First evidence of the emergence of novel putative infectious bronchitis virus genotypes in Cuba.** Acevedo AM, Díaz de Arce H, Brandão PE, Colas M, Oliveira S, Pérez LJ. Res Vet Sci. 2012 Oct;93(2):1046–9.

The emergence of new infectious bronchitis virus (IBV) genotypes or serotypes along with the poor cross-protection observed among IBV serotypes have complicated the avian infectious bronchitis (IB) control programs in different geographic regions. In Cuba, the lack of genetic information regarding IBV and the increasing epidemiological importance of this virus in Cuban chicken flocks demand further characterization of IBV isolates. In the present work, studies of genetic diversity and phylogenetic relationships among recent IBV isolates from Cuban chicken flocks showing respiratory disorders were performed. Two putative genotypes genetically different to the Massachusetts genotype H120 strain used in the Cuban vaccination program were found in the flocks assessed. In addition, a potential nephropathogenic IBV isolate was found by first time in Cuba.

**Gated-SPECT myocardial perfusion imaging and coronary calcium score for evaluation of patients with acute chest pain and a normal or nondiagnostic electrocardiogram.** Peix A, Batista E, Cabrera LO, Rodríguez L, Padrón K, Sainz B, et al. Coron Artery Dis. 2012 Nov;23(7):438–44.

**Objective** To assess the ability of rest myocardial perfusion imaging (MPI) to rule out an acute coronary syndrome (ACS) in emergency department patients, as well as to investigate whether there exists a concordance between MPI and coronary calcium. **Materials and Methods** Fifty-five patients with chest pain and a normal or nondiagnostic ECG were included. Clinical follow-up was carried out within 1 year. **Results** Sixteen patients (29%) showed an abnormal rest MPI, and in 11 (20%) the MPI was equivocal. There was a weak concordance between MPI and coronary arteries calcium score (CACS) ( $\kappa$ : 0.25). Coronary angiogram driven by a positive MPI was performed in 12 patients (23%), resulting in percutaneous coronary intervention in nine cases (75%). A positive MPI (abnormal or equivocal results) was associated with the occurrence of events in the follow-up ( $\chi^2=19.961$ ,  $P<0.0001$ ). For a patient presenting to the emergency department with acute chest pain and a normal or nondiagnostic ECG, with a positive MPI, the relative risk of having events during the first year was 7.5 (95% confidence interval: 2.8–19.2),  $P<0.05$ , but with a positive CACS this was 1.77 (95% confidence interval: 0.69–4.56),  $P=NS$ . At 1 year 68.6% of patients were free of events. **Conclusions** Patients presenting with acute chest pain and a low-to-intermediate likelihood of coronary artery disease with a normal rest MPI have a very low probability of cardiac events during the first year. Coronary calcium score was not helpful in risk-stratifying these patients.

**Granulation Response and Partial Wound Closure Predict Healing in Clinical Trials on Advanced Diabetes Foot Ulcers Treated With Recombinant Human Epidermal Growth Factor.** Valenzuela-Silva CM, Tuero-Iglesias AD, García-Iglesias E, González-Díaz O, Del Río-Martín A, Yera Alos IB, et al. Diabetes Care. 2012 Sep 10. [Epub ahead of print]

**Objective** To determine if partial wound closure surrogate markers proposed for neuropathic, small diabetic foot ulcers (DFUs) can be extended to advanced lesions and if the development of granulation tissue can be used to predict complete healing. **Research Design and Methods** Data from two multicenter, double-blind, randomized clinical trials (one of them placebo controlled) that used intralesional recombinant human epidermal growth factor (rhEGF) to promote granulation and healing were used. For confirmation in a larger sample from common clinical practice, the results of an active postmarketing surveillance of rhEGF treatment of DFUs in 60 healthcare units was included. The surrogates evaluated were percent area change, log healing rate, ratio of log areas, and percent of granulation tissue covering the wound area. The tests used were surrogate final end point correlation, receiver operating characteristic curves to discriminate healers from nonhealers, validation tests using logistic regression models, and the proportion-mediated estimation. **Results** Two weeks >50% granulation, end of treatment >75% granulation, and 16.1% area change

showed significant predictive value (>70% correct classification) for final wound closure. The granulation-based variables fulfilled the criterion that the effect of rhEGF treatment on wound closure was mediated by the surrogate. **Conclusions** This work provides the first evidence for the use of granulation tissue development as a predictor of wound healing in advanced DFUs. These results can be useful for clinical trial design, particularly during the exploratory phase of new products.

**HCV genotype determination in monoinfected and HIV co-infected patients in Cuba.** De los Angeles L, Montalvo MC, Bello M, Sariego S, Marante J, Dueñas S, et al. Trans R Soc Trop Med Hyg. 2012 Dec;106(12):711–7.

With the aim to characterize the HCV genotype distribution in Cuba, sera were collected from two subgroups: HCV-monoinfected and HCV/HIV co-infected patients. A combination of reverse transcription-PCR using genotype-specific primers, restriction fragment length polymorphism and sequencing was used to determine the genotype of 84 samples. Seventy-nine (94%) showed single infections (10 [12%] were genotype 1a and 69 [82%] genotype 1b) and 5 (6%) samples corresponded to mixed infections (2 [2%] with genotypes 1a/3a and 1 sample [1%] each with 1b/3a, 1b/4a and 1a/1b/3a). HCV/HIV co-infected subjects had a higher frequency of mixed infections ( $p=0.08$ ), infection with genotype 3a ( $p=0.18$ ) and for the first time genotype 4a was found. There was no association of any demographic characteristics with any specific genotype although HCV/HIV co-infected patients showed a tendency to have mixed genotypes in those older than 45 years of age ( $p=0.11$ ). Phylogenetic analysis showed that HCV isolates clustered with subtypes 1b ( $n=15$ , maximal genetic distance 2.51%) and 1a ( $n=2$ , maximal genetic distance 0.35%). This report presents the prevalence of HCV genotypes in monoinfected and HIV co-infected patients, mixed HCV infections in HCV/HIV co-infected men who have sex with men with high-risk sexual practices and for the first time identifies that the uncommon genotype 4a can be present in a patient co-infected with HIV.

**High frequency of antiviral drug resistance and non-B subtypes in HIV-1 patients failing antiviral therapy in Cuba.** Kourí V, Alemán Y, Pérez L, Pérez J, Fonseca C, Correa C, et al. J Clin Virol. 2012 Dec;55(4):348–55.

**Background** Emergence of HIV-1 drug resistance may limit the sustained benefits of antiretroviral therapy (ART) in settings with limited laboratory monitoring and drug options. **Objectives** Surveillance of drug resistance and subtypes in HIV-1 patients failing ART in Cuba. **Study design** This study compiled data of ART-experienced HIV-1 patients attending a clinical center in Havana in 2003 and 2009–2011. The first period included results of a cross-sectional study, whereas in the second period genotyp-

ing was performed as part of routine care. Drug resistance mutations and levels were determined using HIVdb version 6.0.9. **Results** Seventy-six percent received solely ART containing at least 3 drugs, of which 79.1% ever receiving unboosted protease inhibitors (PI). Patients from 2009 to 2011 were longer treated and exposed to more ART regimens. Subtype B (39%) and CRF19\_cpx (18%) were the most prevalent genetic forms. Subtype distribution did not change significantly between both periods, except for BG recombinants that increased from 6% to 14%. Nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside RTI (NNRTI) and PI mutations were present in 69.5%, 54.8% and 44.4%. Full-class resistance (FCR) to NRTI, NNRTI, PI and multidrug resistance (MDR) were detected in 31.8%, 37.9%, 18.5% and 15.4%. FCR to NRTI, NNRTI, PI and MDR were present in 9.8%, 14.1%, 0%, 0% after first-line failure and in 19.8%, 20.8%, 2.9% and 2.9% after second-line failure. **Conclusions** Our study found a high prevalence of drug resistance and supports the need for appropriate laboratory monitoring in clinical practice and access to drug options in case of virological failure.

**Intestinal Parasites in Children from a Day Care Centre in Matanzas City, Cuba.** Cañete R, Morales M, Avalos R, Laúd PM, Ponce FM. PLoS One. 2012 Dec 12; e51394. doi:10.1371/journal.pone.0051394.

**Background** Intestinal parasitic infections are widely distributed throughout the world and children are the most affected population. Day care centres are environments where children have proven to be more susceptible to acquiring IP. **Methods and Principal Findings** A cross-sectional study was carried to determine the prevalence of intestinal parasites in stool samples among children who attend to a day care centre in an urban area of Matanzas city, Cuba, from March to June 2012. 104 children under five years old were included on the study after informed consent form was signed by parents or legal guardians. Three fresh faecal samples were collected from each child in different days and were examined by direct wet mount, formalin-ether, and Kato-Katz techniques. Data relating to demography, socioeconomic status, source of drinking water, and personal hygiene habits were also collected using a standardized questionnaire. In total, 71.1% of children harbored at least one type of intestinal parasite and 47 (45.2%) were infected by more than one species. *Giardia duodenalis* and *Blastocystis sp.* were the most common parasites found, with prevalence rates of 54.8% and 38.5% respectively. **Conclusions** Despite public health campaigns, improvement in the level of education, and the availability of and access to medical services in Cuba infections by intestinal protozoan is high in this centre. Almost nothing is published regarding intestinal parasites in Matanzas province during the last 40 years so this work could also be the initial point to carry out other studies to clarify the IP status in this region.

**Kaposi's Sarcoma and Human Herpesvirus 8 in Cuba: evidence of subtype B expansion.** Kourí V, Martínez PA, Capó V, Blanco O, Rodríguez ME, Jiménez N, et al. Virology. 2012 Oct 25;432(2):361–9.

**Objective** To evaluate the temporal distribution (1991-2009) and associated variation of KSHV subtypes in Cuba. **Method** Phylogenetic characterization based on the KSHV K1 gene was performed using 90 KSHV positive samples. **Results** Molecular characterization confirmed the prevalence of a wide range of KSHV subtypes (A: n=48 [A5=12]; C: n=15; B: n=22; and E: n=5). In the current study, we observed a significant increase in HHV-8 subtype B after 2004 ( $p=0.0063$ ). This Subtype B in Cuba was associated with: heterosexual behaviour (OR: 3.63, CI: 1.2-10.98;  $p=0.03$ ), with the antecedent of acquiring HIV/KSHV in Africa ( $p=0.0003$ ), with nodular stage of KS lesions (OR 4.2, CI: 1.1 to 15.7;  $p=0.04$ ). **Conclusions** Our study is the first to report KSHV Subtype B expansion in Cuba, that might be reflective of a change in human behavioural pattern.

**Medium- and Short-Term Interventions with Ma-Pi 2 Macrobiotic Diet in Type 2 Diabetic Adults of Bauta, Havana.** Porrata C, Hernández M, Rodríguez E, Vilá R, Hernández H, Mirabal M, et al. J Nutr Metabol. 2012:1–10.

**Background** In Cuba, the Ma-Pi 2 macrobiotic diet has shown positive results in 6-month assays with type 2 diabetic patients. The objective of this study was to assess the influence of this diet at short and medium terms. **Methods** Sixty-five type 2 diabetic volunteers were included for dietary intervention, institutionally based for 21 days and followed later at home, until completing 3 months. 54 of them stayed until assay end. Before intervention, and after both assay periods, they were submitted to anthropometric records, body composition analyses and measurements of serum biochemical indicators, glycemic profile in capillary blood, blood pressure, and medication consumption; food intake was evaluated by the 3-day dietary recall. **Results** During the intervention, the energy intake was 200 kcal higher at instance of more complex carbohydrates and dietary fiber and despite less fat and protein. Blood pressure and serum biochemical indicators decreased significantly in both periods; the safety nutritional indicators (hemoglobin, serum total proteins, and albumin) showed no variations. The global cardiovascular risk decreased and insulin consumption dropped by 46% and 64%, in both periods, respectively. **Conclusions** The Ma-Pi 2 macrobiotic diet was a successful therapy at short term and after 3-month home-based intervention, for type 2 diabetics.

**Pharmacokinetic study of Growth Hormone-Releasing Peptide 6 (GHRP-6) in nine male healthy volunteers.** Cabrales A, Gil J, Fernández E, Valenzuela C, Hernández F, García I, et al. Eur J Pharm Sci. 2013 Jan 23;48(1–2):40–6.

GHRP-6 is a growth hormone secretagogue that also enhances tissue viability in different organs. In the present work, we studied the pharmacokinetics of this short therapeutic hexapeptide (His-(D-Trp)-Ala-Trp-(D-Phe)-Lys-NH(2,)) MW = 872.44 Da) in nine male healthy volunteers after a single intravenous bolus administration of 100, 200 and 400 µg/kg of body weight. GHRP-6 was quantified in human plasma by a specific LC-MS method, previously developed and validated following FDA guidelines, using (13)C(3)Ala-GHRP-6 as internal standard (Gil et al., 2012, J. Pharmaceutical Biomed. Anal. 60, 19-25). The Lower Limit of Quantification (5 ng/ml) was reached in all subjects at 12 hours post-administration, which was sufficient for modeling a pharmacokinetic profile including over 85% of the Area Under the Curve (AUC). Disposition of GHRP-6 best fitted a bi-exponential function with R(2) higher than 0.99, according to a mathematic modeling and confirmed by an Akaike index (AIC) lower than that of the corresponding one-compartment model for all subjects. Averaging all three dose levels, the distribution and elimination half-life of GHRP-6 were 7.6±1.9 minutes and 2.5±1.1 hours, respectively. These values are coherent with existing data for other drugs whose disposition also fits this model. Dose dependence analysis revealed a noticeable trend for AUC to increase proportionally with administered dose. Atypical GHRP-6 concentration spikes were observed during the elimination phase in four out of the nine subjects studied.

**Setting a colorimetric assay with MTT for assessment of trichomonocidal activity.** Sariego I, Monzote L, Rojas L. Curr Clin Pharmacol. 2012 Nov 7. [Epub ahead of print]

*Trichomonas vaginalis* infection is associated with important problems of public health, including the spreading of other sexual transmitted infections. The existence of clinical resistant isolates to metronidazole and tinidazole, the drugs approved for the treatment of trichomoniasis, points to the necessity of continue searching for trichomonocidal substances. Here we optimize a colorimetric assay with MTT for assess trichomonas viability. The absence of ascorbic acid and cysteine in the culture medium was indispensable to perform the assay, as these compounds spontaneously reduce the MTT. Linearity of absorbance was verified versus trichomonas counts. Medium inhibitory concentration of metronidazole was determined using the sigmoidal Emax model, by comparison the absorbance of test cultures with controls. The obtained value was in the range of published data. The test would be use for the evaluation of trichomonocidal activity of chemical compounds and natural products.

**Species typing of Cuban *Trichomonas vaginalis* virus by RT-PCR, and association of TVV-2 with high parasite adhesion levels and high pathogenicity in patients.** Fraga J, Rojas L, Sariego I, Fernández-Calienes A, Nuñez FA. Arch Virol. 2012 Sep;157(9):1789–95.

The viral infection of the parasite with *T. vaginalis* virus (TVV) may have important implications for trichomonal virulence. In this study we identified the TVV species isolated from Cuban *T. vaginalis*, using specie specific Reverse Transcriptase-PCR. Of the 37 clinical isolates studied, 21 were infected with TVV, 6 contained TVV-1, 12, TVV- 2 and 3 were co-infected with TVV-1 and -2. The strains infected with TVV showing highest adhesion level in comparison to not infected strains, with high statistical significance. The strains infected only with TVV-2 showing highest adhesion level in comparison to strains infected with TVV-1, with high statistical significance. The parasites classified as mild symptomatic are infected only with TVV-1, however the severe only with TVV-2. According to our results, it seems that only two TVV species are infecting the Cuban isolates. Further studies using higher number of strains should be conducted in order to corroborate these results.

### Surveillance of transmitted drug resistance in untreated HIV-infected patients in Cuba.

Machado Zaldivar L, Dubed Echevarría M, Blanco de Armas M, Díaz Torres H, Ruiz Gutierrez N, Romay Franchi D, et al. *JIAS*. 2012 Nov;15 (Suppl 4).

**Background** Knowledge of transmitted drug resistance (TDR) in untreated HIV-infected patients is fundamental in the epidemiological surveillance programs, because it is associated with suboptimal virological outcome of first-line HAART. The aim of this study was to evaluate the epidemiology of TDR in newly diagnosed Cuban patients. **Methods** 250 HIV-1-infected patients diagnosed between 2009 and 2011 were included in the study. RNA was isolated from plasma and used as target to amplify the pol gen by RT-nested PCR. PCR products were sequenced and the data generated used to determine the viral subtype by phylogenetic analyses. The TDR were detected by means of Stanford University calibrated population resistance tool, using the 2009 surveillance drug resistance mutations list. **Results** Baseline characteristic were as follows: 78.4% of the cases were males, mean age was 35.5 years, 66.3% of infections were acquired by homosexual transmission and the median viral load was 4.6 log. The 39.2% of the analyzed samples corresponded to the subtype B and 60.8% for non-B genetic forms, with prevalence of CRF 19\_cpx, CRF 20 BG and CRF 23 BG [1]. The overall prevalence of TDR increased in comparison with previous studies (19.2% versus 5.2%) [2]. The majority of mutations were seen within the group of nucleoside reverse transcriptase (NRTI) (8.5%) and non-NRTI (9.7%). TDR was less common in the group of protease inhibitors (1.7%). The most common mutations were M184V and K103N for the NRTIs and NNRTIs, respectively. The prevalence of TDR in samples of the subtype G was significant ( $p=0.005$ ) in comparison with other genetic forms. **Conclusions** This study confirms an increase in the transmission of resistance-associated muta-

tions, which indicates the importance of maintaining a constant epidemiological surveillance of the TDR in newly diagnosed Cuban patients.

### The Cuban Twin Registry: Initial Findings and Perspectives.

Marcheco B, Cobas M, Cabrera N, Lantigua A, García E, Lardoezt R, et al. *Twin Res Hum Genet*. 2012 Dec 11:1–6.

The Cuban Twin Registry is a nation-wide, prospective, population-based twin registry comprising all zygosity types and ages. It was initiated in 2004 to study genetic and environmental contributions to complex diseases with high morbidity and mortality in the Cuban population. The database contains extensive information from 55,400 twin pairs enrolled in the period 2004–2006. Additionally, 2,600 new multiple births have been included from 2007 to date. In the past 4 years, more than 130 studies have been carried out using the registry with a classical genetic epidemiological approach in which concordance rates for monozygotic and dizygotic twins and heritability of various disease traits were estimated. This article summarizes the history, registry's methodology, recent research findings, and future directions of work.

### The effect of Diamel on patients with metabolic syndrome: a randomised, double-blinded, placebo-controlled study.

Cabrera-Rode E, Orlandi N, Padrón Y, Arranz C, Olano R, Machado M, et al. *J Diabetes*. 2012 Sep 17. doi: 10.1111/1753-0407.12007.

**Objective** The aim of this study was to assess whether the administration of Diamel could improve any of the components of metabolic syndrome (MS), as well as insulin resistance and sensitivity. **Methods** A total of 100 patients with MS, aged between 19 and 70, satisfying the MS criteria established by the WHO, were included in the study. Participants were randomly assigned either oral Diamel or a placebo (while maintaining a diet appropriate to the patient's weight and physical activity), at a dose of two capsules before the three main meals of each day for one year. In addition to anthropometric measures and blood pressure, fasting plasma glucose, lipid profile, insulin, creatinine and uric acid (UA) were determined. Insulin resistance (IR) was assessed. Three indirect indexes were used to calculate insulin sensitivity (IS). **Results** Diamel improved fasting insulin concentrations, IS, IR, and reduced UA concentrations from month six to the end of treatment as compared to placebo ( $P < 0.05$ ). In addition, after the 12(th) month of treatment the only significant changes from baseline in the mean of fasting insulin ( $P < 0.05$ ), UA ( $P < 0.05$ ), IR ( $P < 0.001$ ) and IS ( $P < 0.001$ ) variables occurred in the patients assigned to Diamel, as compared with the patients treated with placebo. The body mass indexes, IR and IS were found to be improved in both groups. **Conclusion** Long-term Diamel treatment, combined with lifestyle changes, was beneficial for insulin resistance,

insulin sensitivity and reduced serum uric acid levels in patients with MS.

### The Natural History of Compensated HCV-Related Cirrhosis; A Prospective Long-Term Study.

Gomez EV, Rodriguez YS, Bertot LC, Gonzalez AT, Perez YM, Soler EA, et al. *J Hepatol*. 2012 Oct 27. pii: S0168-8278(12)00822-7. doi: 10.1016/j.jhep.2012.10.023. [Epub ahead of print]

**Background and aims** The natural history of HCV-related compensated cirrhosis has been poorly investigated in Latin-American countries. Our study evaluated mortality and clinical outcomes in compensated cirrhotic patients followed for 6 years. **Methods** Four hundred and two patients with compensated HCV-related cirrhosis were prospectively recruited in a tertiary care academic center. At the time of admission, patients were stratified as compensated [absence [stage 1], or presence [stage 2] of esophageal varices] as defined by D'Amico et al. Subjects were followed to identify overall mortality or liver transplantation and clinical complication rates. **Results** Among 402 subjects, 294 were categorized as stage 1 and 108 as stage 2. Over a median of 176 weeks, 42 deaths occurred (10%), of which 30 were considered liver-related (7%) and 12 non-liver-related (3%); 8 individuals (2%) underwent liver transplantation; 30 patients (7%) developed HCC, 67 individuals in stage 1 (22%) developed varices and any event of clinical decompensation occurred in 80 patients (20%). The 6-year cumulative overall mortality or liver transplantation was 15% and 45%, for stages 1 and 2, respectively ( $P < 0.001$ ). The cumulative 6-year HCC incidence was significantly higher among patients with varices (29%) than those without varices (9%),  $P < 0.001$ . Similarly, the cumulative 6-year incidence of any clinical liver-related complication was higher in patients with stage 2 (66%) as compared to 26% in those with stage 1, respectively ( $P < 0.001$ ). **Conclusions** Our results indicate significant morbidity and mortality and clinical outcome rates in compensated cirrhotic patients who have varices (stage 2).

### The prevalence, correlates and impact of anaemia among older people in Cuba, Dominican Republic, Mexico, Puerto Rico and Venezuela.

Bryce RM, Salas A, Acosta D, Jiménez IZ, Llibre JJ, Sosa AL, et al. *Br J Haematol*. 2012 Dec 11. doi: 10.1111/bjh.12153. [Epub ahead of print]

Anaemia among older people is increasingly recognized as a matter of public health concern. Data from low- and middle-income countries are sparse. We surveyed 10915 people aged 65 years and over (8423 with blood tests) in catchment areas in Cuba, Dominican Republic, Puerto Rico, Venezuela and Mexico, to assess prevalence and correlates of anaemia and impact on disability. Prevalence varied widely between sites, from 6.4% in rural Mexico to 9.2% in urban Mexico, 9.8% in Ven-

ezueta, 19.2% in Cuba, 32.1% in Puerto Rico and 37.3% in Dominican Republic. Prevalence was higher in men and increased with age, but sociodemographic composition did not account for prevalence differences between sites. Standardized morbidity ratios indicated a much higher prevalence in Cuba (173), Puerto Rico (280) and Dominican Republic (332) compared with USA National Health and National Examination Surveys. Anaemia was associated with under-nutrition, physical impairments, and serum creatinine. There was an association with greater African admixture in Dominican Republic but not in Cuba. African admixture is therefore unlikely to fully explain the high prevalence in the Caribbean islands, which may also arise from envi-

ronmental, possibly dietary factors. Given an important independent contribution of anaemia to disability, more research is needed to identify preventable and treatable causes.

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**Transmitted HIV Type 1 Drug Resistance in Newly Diagnosed Cuban Patients.** Machado LY, Dubed M, Díaz H, Ruiz N, Romay D, Valdés N, et al. AIDS Res Hum Retroviruses. 2012 Oct 25. [Epub ahead of print]

Knowledge of the associated mutations to transmitted drug resistance (TDR) in strains of human immunodeficiency virus type 1 (HIV-1) constitutes a fundamental premise in epidemiological surveil-

lance. In this present study, TDR from 200 Cuban patients who were diagnosed with HIV-1 between 2009 and 2011 was analyzed. By partial reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing of the HIV pol gene, an HIV subtype and transmitted resistance profile were determined. The prevalence of associated mutations to the TDR in the individuals studied was 21.5%. In the region of the reverse transcriptase, the most common mutations were K103N and M184V, while in the region of the protease they were L33F and M46L. The results of this study provide evidence of TDR in the Cuban seropositive population and suggest the necessity of making resistance assays before beginning antiretroviral therapy in HIV-1-infected patients in Cuba. 