

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.

CIGB-300, a synthetic peptide-based drug that targets the CK2 phosphoacceptor domain. Translational and clinical research. Perea SE, Baladron I, Garcia Y, Perera Y, Lopez A, Soriano JL. *Mol Cell Biochem*. 2011 Oct;356(1–2):45–50.

CK2 represents an oncology target scientifically validated. However, clinical research with inhibitors of the CK2-mediated phosphorylation event is still insufficient to recognize it as a clinically validated target. CIGB-300, an investigational peptide-based drug that targets the phosphoacceptor site, binds to a CK2 substrate array in vitro but mainly to B23/nucleophosmin in vivo. The CIGB-300 proapoptotic effect is preceded by its nucleolar localization, inhibition of the CK2-mediated phosphorylation on B23/nucleophosmin and nucleolar disassembly. Importantly, CIGB-300 shifted a protein array linked to apoptosis, ribosome biogenesis, cell proliferation, glycolysis, and cell motility in proteomic studies which helped to understand its mechanism of action. In the clinical ground, CIGB-300 has proved to be safe and well tolerated in a First-in-Human trial in women with cervical malignancies who also experienced signs of clinical benefit. In a second Phase 1 clinical trial in women with cervical cancer stage IB2/II, the MTD and DLT have been also identified in the clinical setting. Interestingly, in cervical tumors the B23/nucleophosmin protein levels were significantly reduced after CIGB-300 treatment at the nucleus compartment. In addition, expanded use of CIGB-300 in case studies has evidenced antitumor activity when administered as compassionate option. Collectively, our data outline important clues on translational and clinical research from this novel peptide-based drug reinforcing its perspectives to treat cancer and paving the way to validate CK2 as a promising target in oncology.

Costs of dengue prevention and incremental cost of dengue outbreak control in Guanatanamo, Cuba. Baly A, Toledo ME, Rodríguez K, Benítez JR, Rodríguez M, Boelaert M, et al. *Trop Med Int Health*. 2011 Sep 12. [Epub ahead of print].

Objective To assess the economic cost of routine *Aedes aegypti* control in an at-risk environment without dengue endemicity and the incremental costs incurred during a sporadic outbreak. **Methods** The study was conducted in 2006 in the city of Guanatanamo, Cuba. We took a societal perspective to calculate costs in months without dengue transmission (January–July) and during an outbreak (August–December). Data sources were bookkeeping records,

direct observations and interviews. **Results** The total economic cost per inhabitant (p.i.) per month. (p.m.) increased from 2.76 USD in months without dengue transmission to 6.05 USD during an outbreak. In months without transmission, the routine *Aedes* control programme cost 1.67 USD p.i. p.m. Incremental costs during the outbreak were mainly incurred by the population and the primary/secondary level of the healthcare system, hardly by the vector control programme (1.64, 1.44 and 0.21 USD increment p.i. p.m., respectively). The total cost for managing a hospitalized suspected dengue case was 296.60 USD (62.0% direct medical, 9.0% direct non-medical and 29.0% indirect costs). In both periods, the main cost drivers for the *Aedes* control programme, the healthcare system and the community were the value of personnel and volunteer time or productivity losses. **Conclusions** Intensive efforts to keep *A. aegypti* infestation low entail important economic costs for society. When a dengue outbreak does occur eventually, costs increase sharply. In-depth studies should assess which mix of activities and actors could maximize the effectiveness and cost-effectiveness of routine *Aedes* control and dengue prevention.

Effectiveness of adaptive designs for phase II cancer trials. López MF, Dupuy JF, González CV. *Contemp Clin Trials*. 2011 Oct 7. [Epub ahead of print].

Background Evaluation of new therapies for cancer has suffered a paradigm shift in the last years. The use of innovative and more efficient designs is a priority for the scientific community; nevertheless, the use of this kind of design is not yet wide spread. **Purpose** In this paper will examine the effectiveness of adaptive designs compared with traditional designs in phase II clinical trials. **Methods** We reviewed a group of abstracts records between 1980 and 2008 and extracted data regarding statistical design, year of publication, kind of evaluated product, localization, sample size and results of the trials. **Results** Nine hundred and eighty-nine clinical trials were identified and from them 333 traditional designs and 19 adaptive designs were included in the review. Two hundred statistical papers were located and 16 were included in the review. The most frequent designs were Standard up and down designs, continual reassessment methods and its variation and designs with Bayesian approaches. More than 80% of the studies evaluated different schemes of chemotherapy. Adaptive designs evaluated only drugs and not any kind of treatment combination and the most often localizations evaluated in both designs were lung, haematology malignan-

cies, and colon cancers. **Conclusions** Adaptive designs are more efficient from the statistical point of view but they are not yet widely used because of complex and computationally intensive methods needed, substantial effort for planning the trials and lack of regulatory guidance.

HIV Type 1 Genetic Diversity in Newly Diagnosed Cuban Patients. Machado Zaldivar LY, Blanco de Armas M, Dubed Echevarría M, Díaz Torres HM, Ruiz Gutiérrez NM, Valdés de Calzadilla N, et al. *AIDS Res Hum Retroviruses*. 2011 Nov 8. [Epub ahead of print].

The knowledge of the genetic diversity of HIV-1 constitutes a fundamental premise in the epidemiological surveillance. In the present study, it was analyzed the HIV-1 genetic variability from 142 Cuban patients who were diagnosed of HIV-1 infection during 2009 and 2010. HIV-1 subtypes were determined by partial RT-PCR and sequencing of HIV-1 pol gene. The phylogenetic analysis showed that 47 (33.1%) samples were subtypes B, and 95 (66.9%) non B subtypes, where G, H and C subtypes, as well as the recombinant forms CRF19_cpx, CRF18_cpx and CRFs BG were included. It was detected for the first time in Cuba the circulation of the CRF05_DF. The analyses of recombinants showed the presence of recombinant CRF18_cpx/CRF19_cpx. The study confirms the high genetic diversity of the HIV-1 and the circulation of new genetic variants in the studied population, which indicates the importance of maintaining a constant epidemiological surveillance in Cuba.

Induction of Immunogenic Apoptosis by Blockade of Epidermal Growth Factor Receptor Activation with a Specific Antibody. Garrido G, Rabasa A, Sánchez B, López MV, Blanco R, López A, et al. *J Immunol*. 2011 Oct 7;187:4954–66.

Despite promising results in the use of anti-epidermal growth factor receptor (EGFR) Abs for cancer therapy, several issues remain to be addressed. An increasing emphasis is being placed on immune effector mechanisms. It has become clear for other Abs directed to tumor targets that their effects involve the adaptive immunity, mainly by the contribution of Fc region-mediated mechanisms. Given the relevance of EGFR signaling for tumor biology, we wonder whether the oncogene inhibition could contribute to Ab-induced vaccine effect. In a mouse model in which 7A7 (an anti-murine EGFR Ab) and AG1478 (an EGFR-tyrosine kinase inhibitor) displayed potent antimeta-

static activities, depletion experiments revealed that only in the case of the Ab, the effect was dependent on CD4(+) and CD8(+) T cells. Correspondingly, 7A7 administration elicited a remarkable tumor-specific CTL response in hosts. Importantly, experiments using 7A7 F(ab')(2) suggested that in vivo Ab-mediated EGFR blockade may play an important role in the linkage with adaptive immunity. Addressing the possible mechanism involved in this effect, we found quantitative and qualitative differences between 7A7 and AG1478-induced apoptosis. EGFR blocking by 7A7 not only prompted a higher proapoptotic effect on tumor metastases compared with AG1478, but also was able to induce apoptosis with immunogenic potential in an Fc-independent manner. As expected, 7A7 but not AG1478 stimulated exposure of danger signals on tumor cells. Subcutaneous injection of 7A7-treated tumor cells induced an antitumor immune response. This is the first report, to our knowledge, of a tumor-specific CTL response generated by Ab-mediated EGFR inhibition, suggesting an important contribution of immunogenic apoptosis to this effect.

Latent tuberculosis infection among health care workers at general hospital in Santiago de Cuba. Borroto S, Gámez D, Díaz D, Martínez Y, Ferrer AI, Velásquez Y, et al. *Int J Tuberc Lung Dis.* 2011 Nov;15(11):1510–5.

Objectives To determine the prevalence of latent tuberculosis infection (LTBI) and its predictors in general hospital personnel. **Methods** Two tuberculin skin tests (TSTs) were carried out within 2 years in a sample of 371 health care workers (HCWs) at a general hospital in Santiago de Cuba Province, along with an interview to explore the association of LTBI with age, sex, occupation, contact history of TB and bacille Calmette-Guérin scar. A 10 mm cut-off point was used; an increase of ≥ 10 mm in the second TST was considered as tuberculin conversion. **Results** Of the 350 HCWs with available TST results, 82% were female; the mean age was 37.6 years. LTBI prevalence was 15.4%: it was highest in professionals (20.6%); 60.3% were non-reactors, and at the second test a year later 1.4% were converters. Among the HCWs, physicians had the highest prevalence (21.8%), followed by nurses (19.6%), while administrative staff had the lowest prevalence (3.3%). The mean induration was 3.78 mm; it was highest in professionals (4.4 mm) and the lowest among support staff (2.6 mm). Contact with TB cases was found to be a risk factor for LTBI. **Conclusions** Even at low levels, TB transmission in this hospital could be associated with risk exposure. It is recommended that preventive chemotherapy be given to tuberculin converters as an infection control measure in this hospital.

Monocyte Activation Test (MAT) reliably detects pyrogens in parenteral formulations of human serum albumin. Perdomo-Morales R, Pardo-Ruiz Z, Spreitzer I, Lagarto A, Montañ T. *ALTEX.* 2011;28(3):227–35.

Disadvantages of the regulatory pyrogen test to assure safety of the end-product Human Serum Albumin (HSA) for parenteral use call for the implementation of an alternative test. In the current study, 16 HSA batches were assayed for pyrogens in parallel with the Rabbit Pyrogen Test, conventional and endotoxin-specific LAL assay and monocyte activation test (MAT). It was found that all HSA batches were contaminated with (1,3)-beta-glucans, which interfere with the conventional LAL. Endotoxin-specific LAL was not suitable to test HSA due to unacceptable endotoxin recovery. Experiments combining polymyxin B and MAT demonstrated that pyrogenic batches were mainly contaminated with endotoxins. However, endotoxin-specific LAL failed to detect one of them. The contaminating (1,3)-beta-glucans enhanced the MAT/IL-6 response to endotoxin, but not that of MAT/IL-1beta. The endotoxin equivalent concentrations obtained using the IL-6 readout were usually higher than those using IL-1beta, probably owing to the direct induction of IL-6 release from monocytes by (1,3)-beta-glucans. The MAT correlates with the rabbit pyrogen test, providing a higher safety level for pyrogenicity testing of HSA and probably other therapeutic proteins.

Safety, immunogenicity and preliminary efficacy of multiple-site vaccination with an Epidermal Growth Factor (EGF) based cancer vaccine in advanced non small cell lung cancer (NSCLC) patients. Rodríguez PC, Neninger E, García B, Popa X, Viada C, Luaces P, et al. *J Immune Based Ther Vaccines.* 2011 Oct 24;9:7.

The prognosis of patients with advanced non small cell lung (NSCLC) cancer remains dismal. Epidermal Growth Factor Receptor is over-expressed in many epithelial derived tumors and its role in the development and progression of NSCLC is widely documented. CimaVax-EGF is a therapeutic cancer vaccine composed by human recombinant Epidermal Growth Factor (EGF) conjugated to a carrier protein, P64K from *Neisseria Meningitidis*. The vaccine is intended to induce antibodies against self EGF that would block EGF-EGFR interaction. CimaVax-EGF has been evaluated so far in more than 1000 advanced NSCLC patients, as second line therapy. Two separate studies were compared to assess the impact of high dose vaccination at multiple anatomic sites in terms of immunogenicity, safety and preliminary efficacy in stage IIIb/IV NSCLC patients. In both clinical trials, patients started vaccination 1 month after finishing first line chemotherapy. Vaccination at 4 sites with 2.4 mg of EGF (high dose) was very safe. The most frequent adverse events were grade 1 or 2 injection site reactions, fever, headache and vomiting. Patients had a trend toward higher antibody response. The percent of very good responders significantly augmented and there was a faster decrease of circulating EGF. All vaccinated patients and those classified as good responders immunized with high dose at 4 sites, had a large tendency to improved survival.

3D Statistical Parametric Mapping of quiet sleep EEG in the first year of life. Bosch Bayard J, Valdés Sosa PA, Fernández T, Otero G, Pliego Rivero B, Ricardo Garcell J, et al. *Neuroimage.* 2011 Nov 10. [Epub ahead of print].

This paper extends previously developed 3D SPM for Electrophysiological Source Imaging (Bosch et al., 2001) for neonate EEG. It builds on a prior paper by our group that established age dependent means and standard deviations for the scalp EEG Broad Band Spectral Parameters of children in the first year of life. We now present developmental equations for the narrow band log spectral power of EEG sources, obtained from a sample of 93 normal neonates from age 1 to 10 months in quiet sleep. The main finding from these regressions is that EEG power from 0.78 to 7.5 Hz decreases with age and also for 45–50 Hz. By contrast, there is an increase with age in the frequency band of 19–32 Hz localized to parietal, temporal and occipital areas. Deviations from the norm were analyzed for normal neonates and 17 with brain damage. The diagnostic accuracy (measured by the area under the ROC curve) of EEG source SPM is 0.80, 0.69 for average reference scalp EEG SPM, and 0.48 for Laplacian EEG SPM. This superior performance of 3D SPM over scalp qEEG suggests that it might be a promising approach for the evaluation of brain damage in the first year of life.

Status of Novel Cardiovascular Risk Factor and Cardiovascular Disease Risk in an Urban Cuban Population—A Pilot Study. Rodríguez Ojea A, Alonso C, Yarnell JW, Woodside JV. *J Health Popul Nutr.* 2011 Oct;29(5):510–5.

Cardiovascular disease is the main cause of death in Cuba, yet the prevalence of novel risk factors is not known. To examine the prevalence of risk factors of traditional and novel cardiovascular diseases (CVDs) among an urban Cuban population, a cross-sectional pilot survey was undertaken in Havana city, Cuba. Ninety-seven adults aged 45–60 years registered to receive medical care at a policlinic. The prevalences of rates of CVD risk factors were: hypertension ($\geq 140/90$ mmHg) (53.6%), hypercholesterolaemia (total cholesterol > 5.2 mmol/L) (47.0%), low high-density lipoprotein (HDL)-cholesterol (< 1.03 mmol/L) (64.3%); diabetes (self-reported) (24.6%); metabolic syndrome (ATP III criteria) (58.2%); overweight and obesity (body mass index ≥ 25 kg/m²) (78.0%); current smoking (39.3%); elevated level of C-reactive protein (3 value < 10 mg/L) (32.1%), low lipid-standardized vitamin E levels (< 5 $\mu\text{mol}/\text{mmol}$ of total cholesterol) (69.6%); and high tHcy levels (> 15 $\mu\text{mol}/\text{L}$) (11.1%). The total carotenoid status was independently associated with waist-circumference and risk of diabetes and metabolic syndrome. In this small unrepresentative sample of people aged 40–65 years from Havana city, there was a high prevalence of traditional and novel CVD risk factors. The total serum carotenoid status appeared to be associated with an increased prevalence of CVD risk factors.

The Association Between Living Through a Prolonged Economic Depression and the Male:Female Birth Ratio--A Longitudinal Study From Cuba, 1960-2008. Venero Fernández SJ, Medina RS, Britton J, Fogarty AW. Am J Epidemiol. 2011 Oct 29;174(12):1327-31.

The Trivers-Willard hypothesis suggests that populations respond to scarcity by decreasing the ratio of males to females at livebirth. Cuba experienced an extreme economic depression in the 1990s called the "special period." Using time-series analysis, the authors studied the impact of this event on the male:female sex ratio at birth in Cuba from 1960 to 2008. From 1990 to 1993, the per capita gross domestic product in Cuba decreased by 36%. By use of a definition of the special period from 1991 to 1998, there was a prolonged increase in the male:female ratio of livebirths during this period of economic depression ($P < 0.001$), from 1.06

at baseline to a peak of 1.18. This association persisted when using alternative definitions of the duration of economic depression in sensitivity analyses. Once the period of economic depression was over, the male:female ratio returned to the baseline value. These data suggest that, in Cuba, contrary to the Trivers-Willard hypothesis, the human population responded to conditions of scarcity by increasing the ratio of males to females at live birth. These data may be relevant in the modeling of demographic projections in countries that experience prolonged economic depression and in understanding adaptive human reproductive responses to environmental change.

Validation of model virus removal and inactivation capacity of an erythropoietin purification process. Pérez M, Rodríguez M, Paez R, Ruibal I, Noa E. Biologicals. 2011 Oct 7.

Human erythropoietin (hEpo) production requires mammalian cells able to make complex post-translational modifications to guaranty its biological activity. As mammalian cell can be reservoir of pathogenic viruses and several animal origin components are usually used in the cultivation of mammalian cells, hEpo contamination with viruses is something of great concern. As consequence, this study investigated the viral removal and inactivation capacity of a recombinant-hEpo (rec-hEpo) purification process. Canine parvovirus, Human poliovirus type-2, Bovine viral diarrhea virus and Human immunodeficiency virus type-1 were used for measuring process viral removal and inactivation capacities. In conclusion, this study corroborated that the assessed rec-hEpo purification process has enough capacity (5.0-19.4 Logs) for removing and inactivating these model viruses and sodium hydroxide demonstrated to be a robust sanitization solution for chromatography columns (5.0 (PV-2)-6.7 (CPV) Logs). 