

Cuban Research in Current International Journals

The following selection – alphabetical by title - reflects Cuban medical publishing in international journals over the last quarter on an array of topics. Links to these journal articles may be found at www.medicc.org/mediccreview.

Antigliadin antibodies in Cuban patients with spinocerebellar ataxia type 2.

Almaguer-Mederos LE, Almira YR, Góngora EM, Gotay DA, Zaldivar YG, Pupo RE et al. *J Neurol Neurosurg Psychiatry*. 2008;79:315-317.

Objective To evaluate the significance of anti-gliadin antibodies (AGA) levels for spinocerebellar ataxia type 2. **Methods** We determined AGA levels in 64 patients with spinocerebellar ataxia type 2 (SCA2) and in 65 healthy matched controls. The clinical assessment was carried out using the International Cooperative Ataxia Rating Scale and CAG repeat number was assessed by PCR. **Results** Antibodies were positive in 23.4% of the ataxia patients and 9.09% of the controls. Statistical comparison using χ^2 test with Yates's correction reveals significant differences between these two groups ($\chi^2 = 3.94$; $p = 0.047$). The same was obtained for strongly positive AGA ($\chi^2 = 4.62$; $p = 0.032$). There were no significant differences between AGA positive and AGA negative patients in age at onset, disease duration, ataxia score or CAG repeat number, neither in the prevalence of gastrointestinal symptoms, prevalence of wheat intolerance or body weight. **Conclusions** These results demonstrate an association between anti-gliadin antibodies serum levels and SCA2. However, more work has to be done to clarify the clinical consequences of such an association.

Biodistribution and internal dosimetry of the 188Re-labelled humanized monoclonal antibody anti-epidermal growth factor receptor, nimotuzumab, in the locoregional treatment of malignant gliomas.

Torres LA, Coca MA, Batista JF, Casaco A, Lopez G, Garcia I, et al. *Nucl Med Commun*. 2008 Jan;29(1):66-75.

Objective To evaluate the biodistribution, internal radiation dosimetry and safety of the 188Re-labelled humanized monoclonal antibody nimotuzumab in the locoregional treatment of malignant gliomas. **Methods** Single doses of 370 or 555 MBq of 188Re-labelled nimotuzumab were locoregionally administered to nine patients with recurrent high-grade gliomas, according to an approved dose-escalation study. SPECT, planar scintigraphy and magnetic resonance images were combined for dosimetric and pharmacokinetic studies. Blood and urine samples were collected to evaluate clinical laboratory parameters and for absorbed doses calculations. Biodistribution, internal dosimetry, human anti-mouse antibody response and toxicity were evaluated and reported. **Results** The 188Re-nimotuzumab showed a high retention in the surgically created resection cavity with a

mean value of $85.5 \pm 10.3\%$ ID 1 h post-injection. It produced mean absorbed doses in the tumour region of approximately 24.1 ± 2.9 Gy in group I (patients receiving 370 MBq) and 31.1 ± 6.4 Gy in group II (patients receiving 555 MBq); the normal organs receiving the highest absorbed doses were the kidneys, liver, and urinary bladder. About $6.2 \pm 0.8\%$ ID was excreted by the urinary pathway. The maximum tolerated dose was 370 MBq because two patients showed severe adverse effects after they received 555 MBq of 188Re-nimotuzumab. No patient developed human anti-mouse antibody response. **Conclusions** A locoregional single dose of 188Re-labelled nimotuzumab of approximately 370 MBq could be used safely in the routine treatment of patients suffering with high-grade gliomas. The efficacy of this therapy needs to be evaluated in a phase II clinical trial.

Classical swine fever virus E2 glycoprotein antigen produced in adenovirally transduced PK-15 cells confers complete protection in pigs upon viral challenge.

Sánchez O, Barrera M, Rodríguez MP, Frías MT, Figueroa NE, Naranjo P, et al. *Vaccine*. 2008 Feb 13;26(7):988-997.

E2 is the major envelope glycoprotein present on the outer surface of the classical swine fever virus (CSFV). It is exposed as a homodimer originated by disulfide linkage and represents an important target for the induction of neutralizing immune responses against the viral infection. The E2his glycoprotein nucleotide sequence used in this work contains the CSFV E2 extracellular domain preceded by the tissue plasminogen signal peptide and a hexahistidine tag in the 3' terminus. The recombinant antigen was produced at a range of 120–150 $\mu\text{g}/\text{mL}$ in the culture media of epithelial kidney pig cells, transduced with a replication defective adenoviral vector (Ad-E2his) generated by means of cloning the E2his sequence in the vector genome. The glycoprotein was obtained from clarified culture media as a homodimer of 110 kDa with purity over 95% after a single affinity chromatography step in Ni-NTA Agarose column. The E2his characterization by lectin-specific binding assay showed the presence of N-linked oligosaccharides of both hybrid and complex types. The protective capacity of E2his was demonstrated in two immunization and challenge experiments in pigs using doses of 15 or 30 μg of the glycoprotein, emulsified in Freund's adjuvant. The intramuscular immunization followed by a unique boost three weeks later, elicited high titers of neutralizing antibodies between the second and the fourth week after the primary vaccination. The immunized animals

were fully protected from the viral infection after challenge with 10^5 PLD₅₀ of homologous CSFV "Margarita" strain administered by intramuscular injection. Consequently, no clinical signs of the disease or viral isolation from lymphocytes were detected in the vaccinated pigs. These results suggest that the E2his antigen produced in mammalian cells may be a feasible vaccine candidate for CSF prevention.

Connecting immunology research to public health: Cuban biotechnology.

Lage A. *Nat Immunol*. 2008 Feb;9(2):109-12.

The close connection between immunology and public health has been successful in reducing infant mortality and infectious diseases in Cuba. The next challenge is to find treatments for chronic diseases of adulthood and to extend life expectancy.

Impact of energy intake, physical activity, and population-wide weight loss on cardiovascular disease and diabetes mortality in Cuba, 1980-2005.

Franco M, Ordúñez P, Caballero B, Tapia Granados JA, Lazo M, Bernal JL, et al. *Am J Epidemiol*. 2007 Dec 15;166(12):1374-80.

Cuba's economic crisis of 1989-2000 resulted in reduced energy intake, increased physical activity, and sustained population-wide weight loss. The authors evaluated the possible association of these factors with mortality trends. Data on per capita daily energy intake, physical activity, weight loss, and smoking were systematically retrieved from national and local surveys. National vital statistics from 1980-2005 were used to assess trends in mortality from diabetes, coronary heart disease, stroke, cancer, and all causes. The crisis reduced per capita daily energy intake from 2,899 calories to 1,863 calories. During the crisis period, the proportion of physically active adults increased from 30% to 67%, and a 1.5-unit shift in the body mass index distribution was observed, along with a change in the distribution of body mass index categories. The prevalence of obesity declined from 14% to 7%, the prevalence of overweight increased 1%, and the prevalence of normal weight increased 4%. During 1997-2002, there were declines in deaths attributed to diabetes (51%), coronary heart disease (35%), stroke (20%), and all causes (18%). An outbreak of neuropathy and a modest increase in the all-cause death rate among the elderly were also observed. These results suggest that population-wide measures designed to reduce energy stores, without affecting nutritional sufficiency,

may lead to declines in diabetes and cardiovascular disease prevalence and mortality.

Intralesional injections of Citoprot-P® (recombinant human epidermal growth factor) in advanced diabetic foot ulcers with risk of amputation.

Fernández-Montequín JI, Infante-Cristiá E, Valenzuela-Silva C, Franco-Pérez N, Savigne-Gutiérrez W, Artaza-Sanz H, et al. *Int Wound J*. 2007 Dec;4(4):333-343(11)

To investigate the efficacy and safety of recombinant human epidermal growth factor (rhEGF) in advanced diabetic foot ulcers (DFU) a double-blind trial was carried out to test two rhEGF dose levels in type 1 or 2 diabetes patients with Wagner's grade 3 or 4 ulcers, with high risk of amputation. Subjects were randomised to receive 75 (group I) or 25 µg (group II) rhEGF through intralesional injections, three times per week for 5-8 weeks together with standardised good wound care. Endpoints were granulation tissue formation, complete healing and need of amputation. Safety was assessed by clinical adverse events (AEs) and laboratory evaluations. Forty-one patients were included. After 5-8 weeks of treatment, 83% patients in the higher dose group and 61% in group II achieved useful granulation tissue covering more than 98% of the wound area. At long-term assessment, 13 (56.5%) patients healed in group I and 9 (50%) in group II. The mean time to complete healing in group I was 20.6 weeks (95% CI: 17.0-24.2) and 19.5 weeks (16.3-22.7) in group II. After 1-year follow-up, only one patient relapsed. Amputation was not necessary in 65% and 66.7% of groups I and II, respectively. The AEs rates were similar. The most frequent were sepsis (33%), burning sensation (29%), tremors, chills and local pain (25% each). rhEGF local injection enhances advanced DFU healing and reduces the risk of major amputation. No dose dependency was observed.

Lack of in vivo embryotoxic and genotoxic activities of orally administered stem bark aqueous extract of *Mangifera indica* L. (Vimang).

González JE, Rodríguez MD, Rodeiro I, Morffi J, Guerra E, Leal F, et al. *Food Chem Toxicol*. 2007 Dec;45(12):2526-32.

Mango (*Mangifera indica* L.) stem bark aqueous extract (MSBE) is a new natural product with antioxidant, anti-inflammatory, and immunomodulatory effects known by the brand name of its formulations as Vimang. Previously, the oral toxicity studies of the extract showed a low toxicity potential up to 2000 mg/kg. This work reports the results about teratogenic and genotoxicologic studies of MSBE. For embryotoxicity study, MSBE (20, 200, or 2000 mg/kg/day) was given to Sprague-Dawley rats by gavage on days 6 to 15 of gestation. For genotoxicity, MSBE was administered three times during 48 h to NMRI mice. Cyclophosphamide (50 mg/kg)

was used as a positive control. No maternal or developmental toxicities were observed when the rats were killed on day 20. The maternal body-weight gain was not affected. No dose-related effects were observed in implantations, fetal viability or external fetal development. Skeletal and visceral development was similar among fetuses from all groups. No genotoxicity was observed in bone marrow erythrocytes and liver cells after administration. MSBE appears to be neither embryotoxic nor genotoxic as measured by bone marrow cytogenetics in rodents.

Lipoprotein NMB0928 from *Neisseria meningitidis* serogroup B as a novel vaccine candidate.

Delgado M, Yero D, Niebla O, González S, Climent Y, Pérez Y, et al. *Vaccine*. 2007 Dec 5;25(50):8420-8431.

Polysaccharide-based vaccines for serogroup B *Neisseria meningitidis* have failed to induce protective immunity. As a result, efforts to develop vaccines for serogroup B meningococcal disease have mostly focused on outer membrane proteins (OMP). Vaccine candidates based on meningococcal OMP have emerged in the form of outer membrane vesicles (OMVs) or, more recently, purified recombinant proteins, as alternative strategies for serogroup B vaccine development. In our group, the protein composition of the Cuban OMVs-based vaccine VA-MENGOC-BC® was elucidated using two-dimensional gel electrophoresis and mass spectrometry. The proteomic map of this product allowed the identification of new putative protective proteins not previously reported as components of an anti-meningococcal vaccine. In the present study, we have determined the immunogenicity and protective capacity of NMB0928, one of those proteins present in the OMVs. The antigen was obtained as a recombinant protein in *Escherichia coli*, purified and used to immunize mice. The antiserum produced against the protein was capable to recognize the natural protein in different meningococcal strains by whole-cell ELISA and Western blotting. After immunization, recombinant NMB0928 induced bactericidal antibodies, and when the protein was administered inserted into liposomes, the elicited antibodies were protective in the infant rat model. These results suggest that NMB0928 is a novel antigen worth to be included in a broadly protective meningococcal vaccine.

Opposite effects of shell or core stimulation of the nucleus accumbens on long-term potentiation in dentate gyrus of anesthetized rats.

López J, Almaguer W, Pérez H, Frey JU, Bergado JA. *Neuroscience*. 2008 Jan 24;151(2):572-578.

Hippocampal long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy which is considered a cellular correlate of learning and memory. It has been shown that both

stimuli with emotional/motivational content and the electrical stimulation [of] the basolateral amygdala can modulate hippocampal LTP. The nucleus accumbens is part of the ventral striatum and is composed of two main regions: core and shell. Core and shell share a similar cellular composition, but differ in their connectivity with other brain areas. Considering that the nucleus accumbens is related to motivation and that it receives a strong projection from the basolateral amygdala, we have studied the effect of stimulating accumbens shell or core on medial perforant path-granule cells' LTP in anesthetized male Wistar rats. We found that electrical stimulation of the shell enhances the magnitude of LTP while the stimulation of the core completely prevents LTP induction. The stimulation of the accumbens shell or core alone produced no apparent, direct field potential in dentate gyrus. Additionally, the co-stimulation of the shell or core with the medial perforant path does not modify the input-output curves obtained using stimulation of the perforant path only. These results demonstrate that electrical stimulation of the accumbens shell or core has a bidirectional effect on LTP induction at the dentate gyrus.

Uses of first line emergency services in Cuba.

De Vos P, Vanlerberghe V, Rodríguez A, García R, Bonet M, Van der Stuyft P. *J Health Policy*. 2008 Jan;85(1):94-104.

Objectives To rationalize the use of hospital emergency units, the Cuban health system developed from 1996 onwards an extra muros first line emergency system (FLES). We analyse the use of the FLES and its determinants, in order to develop proposals to channel inappropriate users to their family doctor. **Methods** In the FLES of an urban (Cerro) and a rural (Baracoa) municipality we collected, from July 1999 to June 2001, data on the moment of consultation, age and sex of the patient, referral status, motive of consultation, emergency classification, diagnosis and medical conduct. A variable "inappropriate use" was constructed. We used multivariate logistic regression to quantify the strength of the associations between patient characteristics, the night-time use, medical procedures, referral, and inappropriate use of the FLES. **Results** Over the two-year observation period, 24,879 and 59,795 patient contacts were registered with the principal emergency policlinic [sic] in Baracoa and Cerro, respectively. In both municipalities the overall "inappropriate" use was almost 60%. There was no correlation with age and gender but inappropriate use was 50% more frequent during the day. Referred patients in both localities were up to 12 times more frequently hospitalized. **Conclusion** Cuba's FLES attracts patients that would be better attended by their family doctor. To strengthen his central position in the health system, one should strengthen the family doctor's technical platform, increase his permanence at the cabinet, and improve communication with the community on the rationale of the family doctor-FLES set up. 