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.

An approach to local immunotoxicity induced by adjuvanted vaccines. Batista-Duharte A, Portuondo D, Carlos IZ, Pérez O. Int Immunopharmacol. 2013 Aug 20;17(3):526–36.

The occurrence of injection site reactions following immunization is the most frequently reported toxicity manifestation of vaccines; however, the different types of local reactions and the different mechanisms involved are still unclear. Here, the current advances in adjuvants and the role that adjuvants play in local reactions are reviewed. The role of adjuvants in the formation of the loco-regional complex (LRC), which consists of the injection site, draining lymphatic vessels and regional lymph nodes, is also discussed. Finally, strategies and recommendations for the rational design of adjuvanted vaccines are discussed, with a particular interest in the reduction of local inflammation.

An Overview of Genetic Counseling in Cuba. Cruz AL. J Genet Couns. 2013 Aug 11. [Epub ahead of print]

This brief report provides an overview of the history and current status of genetic services in Cuba. In 1971, the University of Medical Sciences of Havana began to train doctors in medical genetics according to the medicine development plan in Cuba. With the aim of introducing genetic services to the population, two main issues were identified: the impact of neural tube defects as a cause of infantile mortality, and a founder effect resulting in a high frequency of sickle cell anemia, which increased the mortality rate and impacted the quality of peoples' lives. The impact of consanguinity is variable; it depends on the isolation of the population, with rates of 1 to 11% in different regions for first and second cousin marriages. From 1981, the services of medical genetics began to expand to the entire country, according to a government directive, and the need to design a program for the specialty became evident. From 1995 to 2000, two Masters-level programs were designed by professors of the Department of Medical Genetics, University of Medical Sciences of Havana, and authorized by the Ministry of Higher Education. One program in medical genetics was designed for physicians with other specialties, and the second program was designed to train professionals to become genetic counsellors. The majority of graduates from the latter program are working at the primary level of healthcare.

Brain Tissue Volumes and Perfusion Change with the Number of Optic Neuritis Attacks in Relapsing Neuromyelitis Optica: A Voxel-Based Correlation Study. Sánchez-Catasús CA, Cabrera-Gomez J, Almaguer Melián W, Giroud Benítez JL, Rodríguez Rojas R, Bayard JB, et al. PLoS One. 2013 Jun 18;8(6):e66271.

Recent neuroimaging studies show that brain abnormalities in neuromyelitis optica (NMO) are more frequent than earlier described. Yet, more research considering multiple aspects of NMO is necessary to better understand these abnormalities. A clinical feature of relapsing NMO (RNMO) is that the incremental disability is attack-related. Therefore, association between the attack-related process and neuroimaging might be expected. On the other hand, the immunopathological analysis of NMO lesions has suggested that CNS microvasculature could be an early disease target, which could alter brain perfusion. Brain tissue volume changes accompanying perfusion alteration could also be expected throughout the attack-related process. The aim of this study was to investigate in RNMO patients, by voxel-based correlation analysis, the assumed associations between regional brain white (WMV) and grey matter volumes (GMV) and/or perfusion on one side, and the number of optic neuritis (ON) attacks, myelitis attacks and/or total attacks on the other side. For this purpose, high resolution T1-weighted MRI and perfusion SPECT imaging were obtained in 15 RNMO patients. The results showed negative regional correlations of WMV, GMV and perfusion with the number of ON attacks, involving important components of the visual system, which could be relevant for the comprehension of incremental visual disability in RNMO. We also found positive regional correlation of perfusion with the number of ON attacks, mostly overlapping the brain area where the WMV showed negative correlation. This provides evidence that brain microvasculature is an early disease target and suggests that perfusion alteration could be important in the development of brain structural abnormalities in RNMO.

Comparative Effects of *Roystonea regia* (D-004) and Saw Palmetto Lipid Extracts on Blood Oxidative Variables in Men with Benign Prostate Hyperplasia (BPH). Guzmán R, Illnait J, Mas R, Perez Y, Fernández L, Mendoza L, et al. J Pharmacy. 2013 Aug;3(7):1–8.

Background Lipid extracts of *Roystonea regia* (D-004) and saw palmetto (SP) fruits have been shown to prevent experimentally-induced prostate hyperplasia in rodents, and to produce antioxidant effects in experimental and clinical studies. **Objective** To compare the effects of D-004 and SP extracts on the International Prostate Symptoms Score (IPSS) and plasma oxidative variables in men with benign prostate hyperplasia (BPH). **Methods** This randomized, double-blind study was conducted in patients with moderate BPH. Forty-eight eligible subjects (average age: 65 years) were randomised to D-004 (320 mg/day) or SP (320 mg/day) capsules for 8 weeks. Decrease on IPSS was the primary efficacy variable. Oxidative markers were secondary outcomes. Data were analysed as per Intention to treat. **Results** D-004 and SP significantly decreased mean IPSS values by 33.9% ($p<0.0001$) and 24.4% ($p<0.001$), respectively, as compared to baseline. D-004 ($p<0.0001$) reduced plasma malondialdehyde (MDA) (32.6%), protein-linked carbonyl groups (CG) (25.2%) and increased ($p<0.0001$) catalase (CAT) activity. SP treatment lowered ($p<0.0001$) MDA (28.2%), CG (23.4%) and raised ($p<0.0001$) CAT activity. Effects on oxidative variables were similar in both groups. D-004, not SP, significantly lowered ($p<0.05$) prostate specific antigen (PSA) values. Both treatments were well tolerated. Only 2 SP-treated patients withdrew from the study. No adverse experiences were reported. **Conclusions** Treatment with D-004 or SP (320 mg/day) for 8 weeks decreased significantly IPSS values in patients with moderate BPH, the effect of D-004 being the better, but further studies should confirm this result. Both treatments favourably and similarly modified plasma MDA (lipid peroxidation marker), GC (protein oxidation marker) and CAT activity.

De Novo Mutations in Ataxin-2 Gene and ALS Risk. Laffita-Mesa JM, Rodríguez Pupo JM, Moreno Sera R, Vázquez Mojena Y, Kourí V, Laguna-Salvia L, et al. PLoS One. 2013 Aug 6;8(8):e70560.

Pathogenic CAG repeat expansion in the ataxin-2 gene (ATXN2) is the genetic cause of spinocerebellar ataxia type 2 (SCA2). Recently, it has been associated with Parkinsonism and increased genetic risk for amyotrophic lateral sclerosis (ALS). Here we report the association of de novo mutations in ATXN2 with autosomal dominant ALS. These findings support our previous conjectures based on population studies on the role of large normal ATXN2 alleles as the source for new mutations being involved in neurodegenerative pathologies associated with CAG expansions. The de novo mutations expanded from ALS/SCA2 non-risk alleles as proven by meta-analysis method. The ALS risk was associated with SCA2 alleles as well as with intermediate CAG lengths in the ATXN2. Higher

risk for ALS was associated with pathogenic CAG repeat as revealed by meta-analysis.

Effects of D-002, a mixture of high molecular weight beeswax alcohols, on patients with nonalcoholic fatty liver disease. Illnait J, Rodríguez I, Mendoza S, Fernández Y, Mas R, Miranda M, et al. Korean J Intern Med. 2013 Jul;28(4):439–48.

Background/Aims Nonalcoholic fatty liver disease (NAFLD) is intimately related to insulin resistance and ranges from a benign course to liver fibrosis and cirrhosis. NAFLD management mainly involves dietary modification and weight loss. Although no fully successful pharmacological intervention is available, alternative therapies to treat NAFLD have shown promising results. Experimental studies have shown that D-002, a mixture of beeswax alcohols with antioxidant effects, is hepatoprotective. The aim of this study was to investigate the efficacy and safety of D-002 in patients with NALFD. **Methods** Fifty patients with NAFLD were randomized to receive a placebo or D-002 (100 mg/day) for 24 weeks. The primary endpoint was a significant ultrasonography-detected reduction of liver fat infiltration versus a placebo. Secondary endpoints were decreases in the homeostatic model assessment (HOMA) index, insulin levels, serum liver enzymes, increases in plasma total antioxidant status (TAS) and improved clinical symptoms versus the placebo recipients. **Results** At randomization, all indicators were comparable in both groups. At study completion, seven (28.0%) D-002-patients, but none of the placebo recipients, exhibited a normal liver echo pattern on ultrasonography ($p < 0.01$). Also, D-002 significantly reduced ($p < 0.01$ vs. baseline and placebo) the HOMA index and insulin levels and increased the TAS, but did not affect other parameters. The proportion of D-002-patients (12/25, 48.0%) showing symptom improvement was higher ($p < 0.001$) than that of the placebo group (1/25, 4.0%). The treatment was safe and well tolerated. Three patients in each group withdrew from the study. **Conclusions** D-002 (100 mg/day) improved ultrasonographic findings, indicators of insulin resistance, plasma TAS and clinical evolution on NAFLD patients. Further studies, however, are needed to confirm these results.

Four monoclonal antibodies against capsular polysaccharides of *Neisseria meningitidis* serogroups A, C, Y and W135: Its application in identity tests. Reyes F, Amin N, Otero O, Aguilar A, Cuello M, Valdés Y, et al. Biologicals. 2013 Jul;41(4):275–8. doi: 10.1016/j.biologicals.2013.05.002. Epub 2013 Jun 20.

Murine hybridoma monoclonal antibodies (MAbs) were produced against the capsular polysaccharide (CPs) of serogroups A, C, W135 and Y meningococci (MenA, MenC, MenW, MenY) in order to develop immunological reagents for the identification of meningococcal polysaccharides. Each serogroup-specific MAb reacted with the CPs from its homologous serogroup only and did not react with CPs from the other three serogroups. The affinity constant (Ka) of the four MAbs measured by non-competitive ELISA was 6.62 × 10(9), 2.76 × 10(9), 1.48 × 10(9) and 3.8 × 10(9) M(-1) for MenA, MenC, MenW and MenY MAbs respectively. The application of these MAbs for identity tests was demonstrated by their abilities to correctly identify the CPs from serogroups A, C, W135 and Y in meningococcal CPs-based vaccines through ELISA. The MAbs obtained in this work are a very valuable set of tools for study meningococcal polysaccharides vaccines.

Genetic Features of Huntington Disease in Cuban Population: Implications for phenotype, epidemiology and predictive testing. Vázquez–Mojena Y, Laguna–Salvia L, Laffita–Mesa JM, González–Zaldivar Y, Almaguer–Mederos LE, Rodríguez–Labrada R, et al. J Neurol Sci. 2013 Sep 3. [Epub ahead of print].

Huntington disease is the most frequent polyglutamine disorder with variable worldwide prevalence. Although some Latin American populations have been studied, HD prevalence in Cuban population remains unknown. In order to characterize the disease in Cuba, the relative frequency of HD was determined by studying 130 patients with chorea and 63 unrelated healthy controls, emphasizing in the molecular epidemiology of the disease. Sixty-two patients with chorea belonging to 16 unrelated families carried a pathological CAG expansion in the HTT gene, ranging from 39 to 67 repeats. Eighty-three percent of them come from the eastern region of the country. A significant inverse correlation between age at onset and expanded CAG repeats was seen. Intermediate alleles in affected individuals and controls represented 4.8% and 3.97% respectively, this being this a putative source of de novo mutation. This study represents the largest molecular characterization of Huntington Disease in the Cuban population. These results may have significant implications for an understanding of the disease, its diagnosis and prognosis in Cuban patients, giving health professionals the tools to implement confirmatory genetic testing, pre symptomatic testing and clinical trials in this population.

Immunotherapy–2072. Therapeutic effect and higher safety profile for allergic asthma in Cuban patients with sublingual immunmotherapy using tropical domestic mite allergen vaccines. Castro RL, Alvarez M, Ronquillo M, González M, Labrada A, Rodríguez JS, et al. World Allergy Organ J. 2013;6(Suppl 1):154.

Background Subcutaneous allergen-specific immunotherapy is burdened with the risk of severe systemic reactions; therefore, sublingual administration route has been increasingly investigated worldwide. The goal was to assess the therapeutic effect and safety of allergen therapeutic vaccines of *Dermatophagoides pteronyssinus*, *Dermatophagoides siboney* and *Blomia tropicalis* house-dust mites (VALERGEN, BIOCEN, Cuba) by sublingual route, in asthmatic patients. **Methods** Three Double-Blind Placebo-Controlled clinical trials were performed in 40 patients each, showing asthmatic symptoms and positive predominant Skin Prick Test (SPT) to each mite, respectively. Half of subjects were randomized to placebo. Patients received the treatment consisting on sublingual drops with increasing daily doses for 3 weeks and maintenance doses (2000 BU) twice a week until 12 moths. Therapeutic effect was assessed after 6 and 12 months using symptoms/medication diary cards, peak expiratory flow (PEF) measures and skin sensitivity to investigated mites. Adverse reactions were classified using the World Allergy Organization scale. **Results** The treatment reduced significantly ($p<0.01$) clinical symptoms (38%, CI95%: 33-44) and medication intake (26%, CI95%:21-32) with respect to placebo. The skin sensitivity to the allergens decreased also significantly ($p<0.01$). The allergen amount needed to induce a positive SPT increased 52-fold. PEF variability decreased also significantly ($p<0.05$). The treatment was considered effective in 77% of patients. A major advantage as compared to subcutaneous route was a remarked lower frequency of adverse effects. Local reactions were noted only in 0.43% of administrations. No systemic reactions were observed. **Conclusions** Summarizing sublingual immunotherapy using VALERGEN vaccines is effective and safe in mite-sensitive asthmatic patients.

In vitro anticancer effect of venom from Cuban scorpion *Rhopalurus junceus* against a panel of human cancer cell lines. Díaz–García A, Morier–Díaz L, Frión–Herrera Y, Rodríguez–Sánchez H, Caballero–Lorenzo Y, Mendoza–Llanes D, et al. J Venom Res. 2013 Jun 12;4:5–12. eCollection 2013.

In Cuba the endemic species of scorpion *Rhopalurus junceus* has been used in traditional medicine for cancer treatment. However, there is little scientific evidence about its potential in cancer therapy. The effect of a range of scorpion venom concentrations (0.1, 0.25, 0.5, 0.75 and 1mg/ml) against a panel of human tumor cell lines from epithelial (Hela, SiHa, Hep-2, NCI-H292, A549, MDA-MB-231, MDA-MB-468, HT-29), hematopoietic origins (U937, K562, Raji) and normal cells (MRC-5, MDCK, Vero) was determined by the MTT assay. Additionally, the effect of venom on tumor cell death was assayed by Fluorescence microscopy, RT-PCR and western blot. Only the epithelial cancer cells showed significant cell viability reduction, with medium cytotoxic concentration (IC50) ranging from 0.6-1mg/ml, in a concentration-dependent manner. There was no effect on either normal

or hematopoietic tumor cells. Scorpion venom demonstrated to induce apoptosis in less sensitive tumor cells (Hela) as evidenced by chromatin condensation, over expression of p53 and bax mRNA, down expression of bcl-2 mRNA and increase of activated caspases 3, 8, 9. In most sensitive tumor cells (A549), scorpion venom induced necrosis evidenced by acridine orange/ethidium bromide fluorescent dyes and down-expression of apoptosis-related genes. We concluded the scorpion venom from R. junceus possessed a selective and differential toxicity against epithelial cancer cells. This is the first report related to biological effect of R. junceus venom against a panel of tumor cells lines. All these results make R. junceus venom as a promise natural product for cancer treatment.

Large Normal and Intermediate Alleles in the Context of SCA2 Prenatal Diagnosis. Cruz–Mariño T, Laffita–Mesa JM, González–Zaldívar Y, Velázquez–Santos M, Aguilera–Rodríguez R, Estupiñán-Rodríguez A, et al. J Genet Couns. 2013 Jun 28. [Epub ahead of print]

In 2001 a program for predictive testing of Spinocerebellar Ataxia type 2 was developed in Cuba, based on the detection of an abnormal CAG trinucleotide repeat expansion in the ATXN2 gene. A descriptive study was designed to assess the implications of ATXN2 large normal and intermediate alleles in the context of the SCA2 Prenatal Diagnosis Program. Four clinical scenarios were selected based upon the behaviour of large normal and intermediate alleles when passing from one generation to the next, showing expansions, contractions, or stability in the CAG repeat size. In some populations, traditional Mendelian risk figures of 0 % or 50 % may not be applicable due to the high frequency of unstable large normal alleles. Couples with no family history of SCA2 may have a >0 % risk of having an affected offspring. Similarly, couples in which there is both an expanded and a large normal allele may have a recurrence risk >50 %. It is imperative that these issues be addressed with these couples during genetic counselling. These recurrence risks have to be carefully estimated in the presence of such alleles (particularly alleles ≥27 CAG repeats), carriers need to be aware of the potential risk for their descendants, and programs for prenatal diagnosis must be available for them.

Preclinical modeling of EGFR-specific antibody resistance: oncogenic and immune-associated escape mechanisms. Garrido G, Rabasa A, Garrido C, López A, Chao L, García-Lora AM, et al. Oncogene. 2013 Aug 26. doi: 10.1038/onc.2013.288. [Epub ahead of print]

To define the molecular basis of secondary resistance to epidermal growth factor receptor (EGFR)-specific antibodies is crucial to increase clinical benefit in patients. The limited access to posttreatment tumor samples constitutes the major barrier to conduct these studies, representing preclinical experimentation as a useful alternative. Anti-EGFR antibody-based therapy has been reported to mediate tumor regression by interrupting oncogenic signals and, more recently, by inducing antitumor immunological responses. However, resistance models have been focused only on tumor escape associated with EGFR blockade, whereas studies describing immune-associated escape mechanisms have not been reported thus far. To address this idea, we modelled resistance induction in D122 metastasis-bearing C57BL/6 mice treated with 7A7 (an anti-murine EGFR antibody). Similarly to patients receiving EGFR-specific antibodies, 7A7 resistance promotion represents an important drawback to successful therapy. Characterization of primary cultures derived from metastasis in 7A7-treated mice revealed a high frequency of tumor variants resistant to in vivo and in vitro antibody treatment. We showed, for the first time, the convergence of alterations in oncogenic and immunological pathways in 7A7-resistant variants. To identify key molecules behind resistance, seven 7A7-resistant variants were screened. HER3 overexpression and PTEN deficiency leading to hyperactivation of protumoral downstream signaling were found in these variants as a consequence of 7A7-mediated EGFR inhibition. Concomitantly, we found a high percentage of resistant variants carrying abnormalities in the constitutive and/or interferon gamma (IFN-γ)-inducible major histocompatibility complex I (MHC-I) expression. A significant decrease in mRNA levels for MHC-I heavy chains, β_2-microglogulin and antigen processing machinery genes as well as transcriptional alterations in IFN-γ pathway components were identified as the main mechanisms underlying MHC-I expression defects in 7A7-resistant variants. Notably, these defects have not been previously associated with EGFR-specific antibody resistance, providing novel immunological escape mechanisms. This study has strong implications for the development of new combination strategies to overcome anti-EGFR antibodies refractoriness.

Radiotherapy plus nimotuzumab or placebo in the treatment of high grade glioma patients: results from a randomized, double blind trial. Solomón MT, Selva JC, Figueredo J, Vaquer J, Toledo C, Quintanal N, et al. BMC Cancer. 2013 Jun 19;13(1):299.

Background The prognosis of patients bearing high grade glioma remains dismal. Epidermal Growth Factor Receptor (EGFR) is well validated as a primary contributor of glioma initiation and progression. Nimotuzumab is a humanized monoclonal antibody that recognizes the EGFR extracellular domain and reaches Central Nervous System tumors, in nonclinical and clinical setting. While it has similar activity when compared to other anti-EGFR antibodies, it does not induce skin toxicity or hypomagnesemia. **Methods** A randomized, double blind, multicentric clinical trial was conducted in high grade glioma patients (41 anaplastic astrocytoma and 29 glioblastoma multiforme) that received radiotherapy plus nimotuzumab or placebo. Treatment and placebo groups were well-balanced for the most important prognostic variables. Patients received 6 weekly doses of 200 mg nimotuzumab or placebo together with irradiation as induction therapy. Maintenance treatment was given for 1 year with subsequent doses administered every 3 weeks. The objectives of this study were to assess the comparative overall survival, progression free survival, response rate, immunogenicity and safety. **Results** The median cumulative dose was 3200 mg of nimotuzumab given over a median number of 16 doses. The combination of nimotuzumab and RT was well-tolerated. The most prevalent related adverse reactions included nausea, fever, tremors, anorexia and hepatic test alteration. No anti-idiotypic response was detected, confirming the antibody low immunogenicity. The mean and median survival time for subjects treated with nimotuzumab was 31.06 and 17.76 vs. 21.07 and 12.63 months for the control group. **Conclusions** In this randomized trial, nimotuzumab showed an excellent safety profile and significant survival benefit in combination with irradiation.

Rapid assessment of high-dose radiation exposures through scoring of cell-fusion-induced premature chromosome condensation and ring chromosomes. Lamadrid Boada AI, Romero Aguilera I, Terzouidi GI, González Mesa JE, Pantelias G, García O. Mutat Res. 2013 Jul 12 pii: S1383-5718(13)00191-5. doi: 10.1016/j.mrgentox.2013.06.021. [Epub ahead of print]

Analysis of premature chromosome condensation (PCC) mediated by fusion of G0-lymphocytes with mitotic CHO cells in combination with rapid visualization and quantification of rings (PCC-Rf) is proposed as an alternative technique for dose assessment of radiation-exposed individuals. Isolated lymphocytes or whole blood from six individuals were γ-irradiated with 5, 10, 15 and 20Gy at a dose rate of 0.5Gy/min. Following either 8- or 24-h post-exposure incubation of irradiated samples at 37°C, chromosome spreads were prepared by standard PCC cytogenetic procedures. The protocol for PCC fusion proved to be effective at doses as high as 20Gy, enabling the analysis of ring chromosomes and excess PCC fragments. The ring frequencies remained constant during the 8-24-h repair time; the pooled dose relationship between ring frequency (Y) and dose (D) was linear: $Y=(0.088\pm0.005)\times D$. During the repair time, excess fragments decreased from 0.91 to 0.59 chromatid pieces per Gy, revealing the importance of information about the exact time of exposure for dose assessment on the basis of fragments. Compared with other cytogenetic assays to estimate radiation dose, the PCC-Rf method has the following benefits: a 48-h culture time is not required, allowing a much faster assessment of dose in comparison with conventional scoring of di-centrics and rings in assays for chemically-induced premature chromosome condensation (PCC-Rch), and it allows the

analysis of heavily irradiated lymphocytes that are delayed or never reach mitosis, thus avoiding the problem of saturation at high doses. In conclusion, the use of the PCC fusion assay in conjunction with scoring of rings in G0-lymphocytes offers a suitable alternative for fast dose estimation following accidental exposure to high radiation doses.

Repeated dose toxicity study of *Vibrio cholerae*-loaded gastro-resistant microparticles. López Y, Pastor M, Infante JF, Díaz D, Oliva R, Fernández S, et al. J Microencapsul. 2013 Jun 24. [Epub ahead of print]

Context Microencapsulation of antigens has been extensively studied over the last decades aiming at improving the immunogenicity of vaccine candidates. **Objective** Addressing microparticles (MPs) toxicity in rats. **Material and methods** Spray-dried Eudragit® L 30 D-55 MPs and Eudragit® L 30 D-55 alginate MPs were elaborated and characterized. MPs obtained were administered to rats, three groups were defined: G1, control group; G2, administered with *Vibrio cholerae* (VC)-loaded MPs; G3, receiving VC-loaded alginate MPs. Animals received three vaccine doses. Body weight, food and water intake were controlled during the study. Haematological parameters, vibriocidal titres, organ weight and histology in necropsy were also analyzed. **Results** All animals grew healthy. Body weight gain, food and water intake and haematological parameters remained within physiological values, showing no treatment-related differences. Moreover, organ weight changes were not detected and animals developed protective vibriocidal titres. **Conclusion** VC-loaded MPs and VC-loaded alginate MPs have proved to be safe and effective in the assessed conditions.

Risk factors for wheezing in infants born in Cuba. Venero Fernández SJ, Suárez Medina R, Mora Faife ED, García García G, Valle Infante ID, Gómez Marrero L, et al. QJM. 2013 Aug 7. [Epub ahead of print]

Background Cuba is a unique country, and despite limited economic development has an excellent health system. However, the prevalence of asthma symptoms in children in Havana, Cuba, is unusually high. **Aim** Since early life exposures are critical to the aetiology of asthma, we have studied environmental influences on the risk of wheeze in Cuban infants. **Design** Cross-sectional study. **Methods** A random sample of 2032 children aged 12-15 months living in Havana was selected for inclusion in the cohort. Data were collected using questionnaires administered by researchers. **Results** Of 2032 infants invited to participate, 1956 (96%) infants provided data. The prevalence of any wheeze was 45%, severe wheeze requiring use of the emergency services was 30%, and recurrent wheeze on three or more occasions was 20%. The largest adjusted risk factors for any wheeze were presence of eczema (odds ratio OR 2.09; 95% confidence intervals 95% CI: 1.48-2.94), family history of asthma (OR 2.05; 95% CI: 1.60-2.62), poor ventilation in the home (OR 1.99; 95% CI: 1.48-2.67), attendance at nursery (OR 1.78; 95%CI: 1.24-2.57), male sex (OR1.52; 95% CI: 1.19-1.96) and the number of smokers in the house ($p<0.03$ for trend), OR 1.64 (95%CI: 1.17-2.31) for three or more smokers in home compared to no smokers in the household. **Conclusion** We have identified several risk factors for any wheeze in young infants living in modern day Cuba. As the prevalence of smoking in the home is high (51%), intervention studies are required to determine effective strategies to improve infant health.

Role of the glomerular-tubular imbalance with tubular predominance in the arterial hypertension pathophysiology. Fox MO, Gutiérrez EB. Med Hypotheses. 2013 Sep;81(3):397–9.

In previous investigations we caused renal tubular reabsorption preponderance relating to the glomerular filtration (Glomerular-tubular imbalance) and we observed that this fact conducted to volume expansion and development of arterial hypertension, in rats that previously were normotens. We based on this evidence and other which are reflected in the literature arrived at the following hypothesis: a greater proportion of tubular reabsorption relating to the filtered volume is the base of the establishment of the glomerular-tubular imbalance with tubular predominance (GTI-T), which favors to the Na^+-fluid retention and volume expansion. All of which conduced to arterial hypertension. These facts explain a primary hypertensive role of the kidney, consistent with the results of renal transplants performed in different lines of hypertensive rats and their respective controls and in humans: hypertension can be transferred with the kidney. GTI-T aims to be, a common phenomenon involved in the hypertension development in the multiple ways which is manifested the hypertensive syndrome. In secondary hypertension, GTI-T is caused by significant disruptions of hormone secretions that control renal function, or obvious vascular or parenchymal damage of these organs. In primary hypertension the GTI-T has less obvious causes inherently developed in the kidney, including humoral, cellular and subcellular mechanisms, which may insidiously manifest under environmental factors influence, resulting in insidious development of hypertension. This would explain the state of prehypertension that these individuals suffer. So it has great importance to study GTI-T before the hypertension is established, because when hypertensive state is established, other mechanisms are installed and they contribute to maintain the hypertension. Our hypothesis may explaining the inability of the kidneys to excrete salt and water in hypertension, as Guyton and colleagues have expressed and constitutes a step forward in line with the hypothesis of this author.

Study of Peptide Mimetics of Hepatitis A Virus Conjugated to Keyhole Limpet Hemocyanin and as Multiple Antigen Peptide System. Aguilar A, Camacho F, Martínez R, Huerta V, Garay HE, Amin N, et al. Int J Pept Res Ther. 2013 Aug 23. [Epub ahead of print]

Mimotopes mimic binding properties of natural antigen epitopes. They could be used for vaccine design, drugs development, and diagnostic assays. We have pre-viously identified four bacteriophages displaying hepatitis A virus (HAV) mimotopes from a phage-display peptide library by affinity selection on serum antibodies from hepatitis A patients. Three of these HAV mimotopes showed similarity in their amino acid sequences with at least one of the VP3 and VP1 antigenic proteins of HAV and the four induced specific anti-HAV antibodies. In the present work, four conjugations were done. In each of them, a linear peptide (46, 53, 54 or 56) containing the amino-acid sequence of the corresponding mimotope was conjugated to keyhole limpet hemocyanin (KLH). Conjugation products were named: 46KLH, 53KLH, 54KLH and 56KLH. A two-arm multiple antigen peptide (MAP) system containing peptide sequence 46, and a second MAP containing two copies of peptide sequence 56 were synthesized and dimerized, to obtain the heterodimeric four arms MAP (named MAP46-56) containing two copies of peptides 46 and 56. Mice were immunized with peptides conjugated to KLH and MAP46-56 to evaluate the ability of these two forms of mimotope presentation, to elicit antibodies that bind to the original antigen. KLH conjugated peptides rendered the highest levels of anti-peptide antibodies and were the only ones that induced specific anti-HAV antibodies. The results of immunizations showed that for the mimotopes chosen here, conjugation to a carrier protein was the most effective option to induce antibodies that cross-reacted with the natural antigen.

Understanding the Dengue Viruses and Progress towards Their Control. Rodriguez-Roche R, Gould EA. Biomed Res Int. 2013;2013:690835. doi: 10.1155/2013/690835. Epub 2013 Jul 9.

Traditionally, the four dengue virus serotypes have been associated with fever, rash, and the more severe forms, haemorrhagic fever and shock syndrome. As our knowledge as well as understanding of these viruses increases, we now recognise not only that they are causing increasing numbers of human infections but also that they may cause neurological and other clinical complications, with sequelae or fatal consequences. In this review we attempt to highlight some of these features in the context of dengue virus pathogenesis. We also examine some of the efforts currently underway to control this “scourge” of the tropical and subtropical world.

Use of Strep-tag II for rapid detection and purification of Mycobacterium tuberculosis

recombinant antigens secreted by *Streptomyces lividans*. Ayala JC, Pimienta E, Rodríguez C, Anné J, Vallín C, Milanés MT, et al. J Microbiol Methods. 2013 Jun 17;94(3):192–8.

Recent results with respect to the secretory production of bio-active *Mycobacterium tuberculosis* proteins in Streptomyces have stimulated the further exploitation of this host as a bacterial cell factory. However, the rapid isolation of a recombinant protein by conventional procedures can be a restrictive step. A previous attempt to isolate recombinant antigens fused to the widely used 6His-tag was found to be relatively incompatible with secretory production in the Streptomyces host. As an alternative, the eight-residue Strep-tag® II (WSHPQFEK), displaying intrinsic binding affinity towards streptavidin, was evaluated for the secretory production of two *M. tuberculosis* immunodominant antigens in *Streptomyces lividans* and their subsequent downstream processing. Therefore, the genes ag85A (Rv3804c, encoding the mycolyl-transferase Ag85A) and Rv2626c (encoding hypoxic response protein 1), were equipped with a 3'-Strep-tag® II-encoding sequence and placed under control of the *Streptomyces venezuelae* CBS762.70 subtilisin inhibitor (vsi) transcriptional, translational and signal sequences. Strep-tagged Ag85A and Rv2626c proteins were detected in the spent medium of recombinant *S. lividans* cultures at 48h of growth, and purified using a Strep-Tactin Superflow® matrix. Recombinant Ag85A appeared as a 30-kDa protein of which the N-terminal amino acid sequence was identical to the expected one. Rv2626c was produced in two forms of 17 and 37kDa respectively, both with the same predicted N-terminal sequence, suggesting that the 37-kDa product is an Rv2626c dimer. The obtained results indicate that the Strep-tagII is proteolytically stable in Streptomyces and does not interfere with the membrane translocation of Ag85A and Rv2626c. A comparison of reactivity of serum from tuberculosis patients versus healthy persons by ELISA showed that both S. lividans-derived antigens were recognized by sera of individuals infected with *M. tuberculosis*, indicating that they remained antigenetically active. To our knowledge, this is the first report showing the usefulness of an affinity peptide for detection and efficient downstream processing of recombinant proteins produced in Streptomyces. The present results add up strength to the significance of *S. lividans* as a valuable host to produce *M. tuberculosis* proteins with vaccine and diagnostic potential.

Zolpidem induces paradoxical metabolic and vascular changes in a patient with PVS. Rodríguez-Rojas R, Machado C, Alvarez L, Carballo M, Estévez M, Pérez-Nellar J, et al. Brain Inj. 2013;27(11):1320–9.

Introduction Zolpidem is a non-benzodiazepine drug used for the therapy of insomnia, which has selectivity for stimulating the effect of GABA-A receptors. Recently, a paradoxical arousing effect of zolpidem in patients with severe brain damage has been repeatedly reported. **Methods** A placebo-controlled magnetic resonance study was conducted to evaluate its effect on BOLD and metabolites spectral signals in a patient with severe brain injuries and an age-matched healthy volunteer. A multi-modal analysis was used to assess aspects in the pharmacologically-induced changes in the resting-state brain metabolism. **Results** A significantly increased BOLD signal was transiently localized in the left frontal cortices, bilateral anterior cingulated areas, left thalamus and right head of the caudate nucleus. The healthy subject showed a deactivation of the frontal, parietal and temporal cortices. BOLD signal changes were found to significantly correlate with concentrations of extravascular metabolites in the left frontal cortex. It is discussed that, when zolpidem attaches to modified GABA receptors of neurodormant brain cells, brain activation is induced. This might explain the significant correlations of BOLD signal changes and proton-MRS metabolites in this patient after zolpidem. **Conclusion** It was concluded that proton-MRS and BOLD signal assessment could be used to study zolpidem-induced metabolic modulation in a resting state.