Cuban Research in Current International Journals

A meta-analysis of the efficacy of albendazole compared with tinidazole as treatments for Giardia infections in children.
Escobedo AA, Ballesteros J, González-Fraile E, Almirall P. Acta Trop. 2015 Oct 14. pii: S0001-706X(15)30121-2. DOI: 10.1016/j.actatropica.2015.09.023. [Epub ahead of print]

Metronidazole is frequently used against this infection; however, it has been associated with significant failure rates in clearing parasites from the gut; additionally, as it should be taken for 5 to 10 days, it is associated with poor compliance, probably due to side effects. Other drugs, including tinidazole (TNZ) and albendazole (ABZ) have been included in the antigiardial armamentarium. Our aim was to assess the efficacy of ABZ compared with TNZ in Giardia infections in children. A systematic review and a meta-analysis were carried out. PubMed, Medline, EMBASE, CENTRAL, and LILACS were searched electronically until February 2015. Also relevant journals and references of studies included therein were hand-searched for randomised controlled trials (RCTs). The meta-analysis was limited to RCTs evaluating the use of ABZ compared with TNZ in children with Giardia infection. The assessed outcome was parasitological efficacy. Prediction intervals (PI) were computed to better express uncertainties in the effect estimates. Five RCTs including 403 children were included. Overall, TNZ significantly outperformed ABZ without differences between subgroups defined by ABZ dosages [relative risk, (RR) 1.61 (95% CI: (1.40 to 1.85); P<0.0001]. The 95% prediction interval range is 1.28 - 2.02. There was no significant heterogeneity (I2=0%; Q-test of heterogeneity P=0.4507. The number-needed-to-treat, the average number of patients who need to be treated with TNZ to gain one additional good outcome as compared with ABZ was 4, 95% CI: 3 to 5. Our results show that TNZ outperforms ABZ in the treatment of Giardia infections in children from developing countries.

A Phase III Clinical Trial of the Epidermal Growth Factor Vaccine CIMAvax-EGF as Switch Maintenance Therapy in Advanced Non-Small-Cell Lung Cancer Patients.
Rodríguez PC, Popa X, Martínez O, Mendoza S, Santiesteban E, Crespo T, et al. Clin Cancer Res. 2016 Feb 29. pii: clincanres.0855.2015. [Epub ahead of print]

Purpose Epidermal Growth Factor Receptor (EGFR) is a well validated target for Non-Small-Cell-Lung-Cancer (NSCLC) patients. CIMAvax-EGF is a therapeutic cancervaccine composed by human-recombinant EGF conjugated to a carrier protein and Montanide ISA51, as adjuvant. The vaccine is intended to induce antibodies against self EGF that block EGF-EGFR interaction. **Experimental design**To evaluate overall survival, safety, immunogenicity and EGF concentration in serum after CIMAvax-EGF, a randomized phase III trial was done in advanced NSCLC patients. Four to 6 weeks after first-line chemotherapy, 405 stage IIIB/IV NSCLC patients were randomly assigned to a vaccine group, which received CIMAvax-EGF or a control group, treated with best supportive care. **Results** These differences were not significant according the standard log-rank (HR 0.82; p=0.100) but according a weighted log-rank (p=0.04), that was applied once the non-proportionality of the hazard ratio was verified. Survival benefit was significant (HR 0.77; p=0.036) in the per-protocol setting (patients receiving at least 4 vaccine doses): MST was 12.43 months for the vaccine arm vs. 9.43 months for the control arm. MST was larger (14.66 months) for vaccinated patients with high EGF concentration at baseline. **Conclusions**Switchmaintenance with CIMAvax-EGF was well tolerated and significantly increased MST of patients that completed induction vaccination. Baseline EGF concentration predicted survival benefit.

Arresting progressive atherosclerosis by immunization with an anti-glycosaminoglycan monoclonal antibody in apolipoprotein E-deficient mice.
Delgado-Roche L, Brito V, Acosta E, Pérez A, Fernández JR, Hernández-Matos Y, et al.
Free Radic Biol Med. 2015 Oct 8;89:557–66. DOI: 10.1016/j.freeradbiomed.2015.08.027. [Epub ahead of print]

Atherogenesis is associated with the early retention of low-density lipoproteins (LDL) in the arterial intima by interaction with glycosaminoglycan (GAG)-side chains of proteoglycans. Retained LDL undergo reactive oxygen species-mediated oxidation. Oxidized LDL trigger oxidative stress (OS) and inflammation, contributing to atherosclerosis development. Recently, we reported the preventive anti-atherogenic properties of the chimeric mouse/human monoclonal antibody (mAb) chP3R99-LALA, which were related to the induction of anti-chondroitin sulfate antibody response able to inhibit chondroitin sulfate dependent LDL-enhanced oxidation. In the present work, we aimed at further investigating the impact of chP3R99-LALA mAb vaccination on progressive atherosclerosis in apolipoprotein E-deficient mice (apoE-/-) fed with a high-fat high-cholesterol diet receiving 5 doses (50μg) of the antibody subcutaneously, when ~5% of the aortic area was covered by lesions. Therapeutic immunization with chP3R99-LALA mAb halted atherosclerotic lesions progression. In addition, aortic OS was modulated, as shown by a significant (p<0.05) reduction of lipid and protein oxidation,

preservation of antioxidant enzymes activity and reduced glutathione, together with a decrease of nitric oxide levels. chP3R99-LALA mAb immunization also regulated aortic NF-κB activation, diminishing the proinflammatory IL1-β and TNF-α gene expression as well as the infiltration of macrophages into the arterial wall. The therapeutic immunization of apoE-/- with progressive atheromas and persistent hypercholesterolemia using chP3R99-LALA mAb arrested further development of lesions, accompanied by a decrease of aortic OS and NF-κB-regulated pro-inflammatory cytokine gene expression. These results contribute to broaden the potential use of this anti-GAG antibody-based immunotherapy as a novel approach to target atherosclerosis at different phases of progression.

Assessment of the FasciMol-ELISA in the detection of the trematodeFasciola hepatica in field-collected Galba cubensis: a novel tool for the malacological survey of fasciolosis transmission.
Alba A, Vázquez AA, Sánchez J, Fraga J, Hernández H, Martínez E, et al. Parasit Vectors. 2016 Jan 16;9(1):22.
Background *Fasciolosis* is one of the food-borne neglected trematodioses that has reemerged as a human disease while its effects on domestic animal health remains of significant economic consideration. Being snail-borne disease, the accurate and time-saving epidemiological surveillance of the transmission foci where infected lymnaeid snails occur could be essential to effectively focus or redirect control strategies. For this purpose, the first monoclonal antibody-based immunoenzymatic assay to detect Fasciolahepatica-infected snails (FasciMol-ELISA) was recently developed and showed a high sensitivity and specificity when tested in an experimental F. hepatica - Galbacubensis system.**Methods** Here, we surveyed populations of G. cubensis occurring in western Cuba for the assessment of the FasciMol-ELISA in determining natural F. hepatica infection in this intermediate host. A multiplex PCR, previously developed to detect F. hepatica in G. cubensis, was used for sample classification. Snail dissection method was also employed as screening technique. A X (2) test and a Kappa index were calculated to evaluate the positivity and the level of agreement between the FasciMol-ELISA and the snail dissection methods with the multiplex PCR, respectively. **Results**Galbacubensis was found in nine out of 12 sampled localities of which four were positive for F. hepatica infection as detected by both immunoenzymatic and PCR-based assays. The overall prevalence was higher than the natural infection rates previously reported for Cuban G. cubensis (range from 4.1 to 7.42 % depending on the screening method). No significant differences were found between FasciMol-ELISA and multiplex PCR when determining parasite positivity (X (2) = 6.283; P = 0.0981) whereas an excellent agreement was also noted (Kappa = 0.8224). **Conclusions** Our results demonstrate the importance of malacological surveys in assessing parasite transmission risk and constitute an alert on the need of accurate measures to control *fasciolosis* in western Cuba. The sensitivity and specificity of the FasciMol-ELISA as well as its time-saving capacity and the easy of performing the determination of a large number of samples, point at this assay as a noveltool suitable for large-scale monitoring of natural snails populations. To our knowledge, this is the first study that explores natural infection by F. hepatica in field-occurring lymnaeid snails using an immunoenzymatic assay.

Efficient and biologically relevant consensus strategy for Parkinson's disease gene prioritization.
Cruz-Monteagudo M, Borges F, Paz-Y-Miño C, Cordeiro MN, Rebelo I, Pérez-Castillo Y, et al. BMC Med Genomics. 2016 Mar 9;9(1):12.

Background The systemic information enclosed in microarray data encodes relevant clues to overcome the poorly understood combination of genetic and environmental factors in Parkinson's disease (PD), which represents the major obstacle to understand its pathogenesis and to develop disease-modifying therapeutics. While several gene prioritization approaches have been proposed, none dominate over the rest. Instead, hybrid approaches seem to outperform individual approaches. **Methods** A consensus strategy is proposed for PD related gene prioritization from mRNA microarray data based on the combination of three independent prioritization approaches: Limma, machine learning, and weighted gene co-expression networks. **Results** The consensus strategy outperformed the individual approaches in terms of statistical significance, overall enrichment and early recognition ability. In addition to a significant biological relevance, the set of 50 genes prioritized exhibited an excellent early recognition ability (6 of the top 10 genes are directly associated with PD). 40 % of the prioritized genes were previously associated with PD including well-known PD related genes such as SLC18A2, TH or DRD2. Eight genes (CCNH, DLK1, PCDH8, SLIT1, DLD, PBX1, INSM1, and BMI1) were found to be significantly associated to biological process affected in PD, representing potentially novel PD biomarkers or therapeutic targets. Additionally, several metrics of standard use in chemoinformatics are proposed to evaluate the early recognition ability of gene prioritization tools. **Conclusions** The proposed consensus strategy represents an efficient and biologically relevant approach for gene prioritization tasks

providing a valuable decision-making tool for the study of PD pathogenesis and the development of disease-modifying PD therapeutics.

Exogenous surfactant and alveolar recruitment in the treatment of the acute respiratory distress syndrome.
Rodríguez-Moya VS, Gallo-Borrero CM, Santos-Áreas D, Prince-Martínez IA, Díaz-Casañas E, López-Herce Cid J. Clin Respir J. 2016 Feb 16. DOI: 10.1111/crj.12462. [Epub ahead of print]

Objective To investigate the effect of alveolar recruitment combined with surfactant administration on children with acute respiratory distress syndrome (ARDS). **Material and methods** A prospective, randomized, controlled and sequential study was carried out. Group A (16 children) was treated with both the alveolar recruitment manoeuvres and the administration of the surfactant every 8 hours for three days; group B (15) received the usual treatment only. The alveolar recruitment was carried out by increasing PEEP 2 by 2 cm H2 O to improve the transcutaneous oxygen saturation values up to 88% and 90%. Demographic data, gasometric and ventilator parameters, chest radiography, and 28-day mortality were evaluated.**Results** There were no significant differences in baseline characteristics between groups. An hour after treatment, significant differences (p<0.001) were observed in SaO2 (Group A: 94.1%, Group B: 89.9%), PaO2 /FiO2 (212.7 and 126.4), and OI (11.4 and 18.5). After 8 hours, the differences in SaO2 (Group A: 94.6%, Group B: 90.3%), PaO2 /FiO2 (225.8 and 126.9), and OI (10.8 and 18.4) were also significant (p<0,001). From the 5th dose of the surfactant, the static compliance (p=0.0034) and radiological images (p=0.002) were more greatly improved in group A than in group B. Survival was significantly higher in group A (81.3%) than in group B (26.7%) (p=0.006).**Conclusions**The combined treatment of surfactant administration and alveolar recruitment manoeuvres resulted in a better oxygenation and survival in children with ARDS than when only recruitment was used.

Exploring the Effectiveness of External Use of Bach Flower Remedies on Carpal Tunnel Syndrome: A Pilot Study.
Rivas-Suárez SR, Águila-Vázquez J, Suárez-Rodríguez B, Vázquez-León L, Casanova-Giral M, Morales-Morales R, et al. J Evid Based Complementary Altern Med. 2015 Oct 11. pii: 2156587215610705. [Epub ahead of print]

Background A randomized, pilot, placebo-controlled clinical trial was conducted with the aim of evaluating the effectiveness of a cream based on Bach flower remedies (BFR) on symptoms and signs of carpal tunnel syndrome. **Methods** Forty-three patients with mild to moderate carpal tunnel syndrome during their "waiting" time for surgical option were randomized into 3 parallel groups: Placebo (n = 14), blinded BFR (n = 16), and nonblinded BFR (n = 13). These groups were treated during 21 days with topical placebo or a cream based on BFR. **Results** Significant improvements were observed on self-reported symptom severity and pain intensity favorable to BFR groups with large effect sizes (η2 partial > 0.40). In addition, all signs observed during the clinical exam showed significant improvements among the groups as well as symptoms of pain, night pain, and tingling, also with large effect sizes (φ > 0.5). Finally, there were significant differences between the blinded and nonblinded BFR groups for signs and pain registered in clinical exam but not in self-reports. **Conclusion** The proposed BFR cream could be an effective intervention in the management of mild and moderate carpal tunnel syndrome, reducing the severity symptoms and providing pain relief.

Harmonization of QSAR Best Practices and Molecular Docking Provides an Efficient Virtual Screening Tool for Discovering New G-Quadruplex Ligands.
Castillo-González D, Mergny JL, De Rache A, Pérez-Machado G, Cabrera-Pérez MA, Nicolotti O, et al. J Chem Inf Model. 2015 Oct 26;55(10):2094–110. DOI: 10.1021/acs.jcim.5b00415. Epub 2015 Sep 18.

Telomeres and telomerase are key players in tumorogenesis. Among the various strategies proposed for telomerase inhibition or telomere uncapping, the stabilization of telomericG-quadruplex (G4) structures is a very promising one. Additionally, G4 stabilizing ligands also act over tumors mediated by the alternative elongation of telomeres. Accordingly, the discovery of novel compounds able to act on telomeres and/or inhibit the telomerase enzyme by stabilizing DNA telomeric G4 structures as well as the development of approaches efficiently prioritizing such compounds constitute active areas of research in computational medicinal chemistry and anticancer drug discovery. In this direction, we applied a virtualscreening strategy based on the rigorous application of QSARbestpractices and its harmonized integration with structure-based methods. More than 600,000 compounds from commercial databases were screened, the first 99 compounds were prioritized, and 21 commercially available and structurally diverse candidates were purchased and submitted to experimental assays. Such strategy proved to be highly efficient in the prioritization of G4 stabilizer hits, with a hit rate of 23.5%. The best G4 stabilizer hit found exhibited a shift in melting temperature from FRET assay of +7.3 °C at

5 μM, while three other candidates also exhibited a promising stabilizing profile. The two most promising candidates also exhibited a good telomerase inhibitory ability and a mild inhibition of HeLa cells growth. None of these candidates showed antiproliferative effects in normal fibroblasts. Finally, the proposed virtualscreening strategy proved to be a practical and reliable tool for the discovery of novel G4 ligands which can be used as starting points of further optimization campaigns.

Healing enhancement of diabetic wounds by locally infiltrated epidermal growth factor is associated with systemic oxidative stress reduction.
Ojalvo AG, Acosta JB, Marí YM, Mayola MF, Pérez CV, Gutiérrez WS, et al. Int Wound J.2016 Mar 22. DOI: 10.1111/iwj.12592. [Epub ahead of print]

The diabetic foot ulcer (DFU) is the leading cause of lower extremity amputation worldwide and is directly associated with comorbidity, disability and mortality. Oxidative stress mechanisms have been implicated in the pathogenesis of these wounds. Intra-lesional infiltration of epidermal growth factor has emerged as a potential therapeutic alternative to allow for physiological benefit while avoiding the proteolytic environment at the centre of the wound. The aim of this study was to characterise the response of patients with DFUs to epidermal growth factor treatment in terms of redox status markers. Experimental groups included patients with DFUs before and 3–4 weeks after starting treatment with epidermal growth factor; compensated and non-compensated diabetic patients without ulcers; and age-matched non-diabetic subjects. Evaluations comprised serum levels of oxidative stress and antioxidant reserve markers. Patients with DFUs exhibited the most disheveled biochemical profile, with elevated oxidative stress and low antioxidant reserves, with respect to non-ulcerated diabetic patients and to non-diabetic subjects. Epidermal growth factor intra-lesional administration was associated with a significant recovery of oxidative stress and antioxidant reserve markers. Altogether, our results indicate that epidermal growth factor intra-ulcer therapy contributes to restore systemic redox balance in patients with DFUs.

Immunological evaluation of rheumatoid arthritis patients treated with itolizumab.
Aira LE, Hernández P, Prada D, Chico A, Gómez JA, González Z, et al.
MAbs. 2016 Jan;8(1):187–95.

Rheumatoid arthritis is an autoimmune disease characterized by joint inflammation that affects approximately 1% of the general population. Itolizumab, a monoclonal antibody specific for the human CD6 molecule mainly expressed on T lymphocytes, has been shown to inhibit proliferation of T cells and proinflammatory cytokine production in psoriasis patients. We have now assessed the immunological effect of itolizumab in combination with methotrexate in rheumatoid arthritis by analyzing clinical samples taken from 30 patients enrolled in a clinical trial. T and B cell subpopulations were measured at different time points of the study. Plasma cytokine levels and anti-idiotypic antibody response to itolizumab were also evaluated. The combined treatment of itolizumab and methotrexate led to a reduction in the frequency of T cell subpopulations, and plasma levels of proinflammatory cytokines showed a significant decrease up to at least 12 weeks after treatment ended. No anti-idiotypic antibody response was detected. These results support the relevance of the CD6 molecule as a therapeutic target for the treatment of this disease.

ImmunoscintigraphyWith 99mTc-Nimotuzumab for Planning Immunotherapy in Patients With Bone Metastases Due to Prostate Cancer.
Quián YP, Crombet T, Batista JF, Prats A, Perera A.Clin Nucl Med. 2015 Oct 8. [Epub ahead of print]

Detection of bone metastases indicates poor prognosis for patients with prostate cancer. The immunotherapy with monoclonal antibody has been an important advance in the treatment of the cancer in the last years. Nimotuzumab is a humanized IgG1 monoclonal antibody directed against epidermal growth factor receptor that has been evaluated in solid tumors. The authors show images of 2 patients with bone metastases secondary to prostate cancer, "pre-cold therapy" with nimotuzumab. Immunoscintigraphic images were acquired 4 and 24 hours after the intravenous administration of 1110 MBq (30 mCi) of Tc-labeled nimotuzumab. Bone metastases expressing the receptor are visualized.

Impaired glucose metabolism increases risk of hepatic decompensation and death in patients with compensated hepatitis C virus-related cirrhosis.
Calzadilla-Bertot L, Vilar-Gómez E, Torres-González A, Socias-López M, Diago M, Adams LA. Dig Liver Dis. 2016 Mar;48(3):283–90.

Background Glucose metabolism abnormalities frequently coexist with liver cirrhosis; however, the impact of these on liver-related outcomes has not been fully investigated. **Aims** We examined the influence of glucose abnormalities on overall mortality and liver-related complications in cirrhotic patients. **Methods** A prospective cohort of 250 subjects with compensated hepatitis C virus-related cirrhosis and without known diabetes underwent an oral glucose tolerance test and were subsequently followed for a median 201 weeks. **Results** At baseline, 67 (27%) had type 2 diabetes. During follow-up, 28 deaths and 55 first events of decompensation occurred. After adjustment for potential confounding covariates, overall mortality/liver transplant (sHR: 2.2, 95% CI: 1.04-4.6, P=0.04) and hepaticdecompensation events (sHR: 1.9, 95% CI: 1.05-3.3, P=0.03) were significantly higher in diabetic patients. Subjects with a HOMA-IR >5 showed higher rates of mortality (sHR: 2.2, 95% CI: 1.03-4.8, P=0.04). The rates of hepatic decompensation were higher in patients with HOMA-IR >3 (sHR: 1.7, 95% CI: 1.04-2.9, P=0.03). Overall, 2h-plasma glucose was the most robust predictor of overall mortality (sHR: 2.5, 95% CI: 1.03-6, P=0.04) and decompensation (sHR: 2.7, 95% CI: 1.4-5.5, P<0.01).**Conclusions** In compensated HCV-related cirrhotic patients, diabetes and marked insulin resistance are independently associated with poorer overall survival and increased risk of hepatic decompensation.

Increasing clinical severity during a dengue virus type 3 Cuban epidemic: deep sequencing of evolving viral populations.
Rodríguez-Roche R, Blanc H, Bordería AV, Díaz G, Henningsson R, González D, et al. J Virol. 2016 Feb 17.pii: JVI.02647-15. [Epub ahead of print]

During the DENV-3epidemic occurred in Havana in 2001-2002, severe disease was associated with the infection sequence DENV-1/DENV-3, whilst the sequence DENV-2/DENV-3 was associated with mild/asymptomatic infections. To determine the role of the virus in the increasing severity demonstrated during the epidemic serum samples collected at different point times were studied. A total of 22 full-length sequences were obtained using a deep sequencing approach. Bayesian phylogenetic analysis of consensus sequences revealed that two DENV-3 lineages were circulating in Havana at that time, both grouped within genotype III. The predominant lineage is closely related to Peruvian and Ecuadorian strains, whilst the minor lineage is related to Venezuelan strains. According to consensus sequences, relatively few non-synonymous mutations were observed; only one was fixed during the epidemic at position 4380 in the NS2B gene. Intra-host genetic analysis indicated that a significant minor population was selected and became predominant towards the end of the epidemic. In conclusion, greater variability was detected during the epidemic's progression in terms of significant minority variants, particularly in the non-structural genes. An increasing trend of genetic diversity towards the end of the epidemic was only observed for synonymous variant allele rates, with higher variability in secondary cases. Remarkably, significant intra-host genetic variation was demonstrated within the same patient during the course of secondary infection DENV-1/DENV-3, including changes in the structural proteins PrM and E. Therefore, the dynamic of evolving viral populations in the context of heterotypic antibodies could be related to the increasing clinical severity observed during the epidemic.**Importance** Based on the evidence that DENV fitness is context dependent, our research has focused on the study of viral factors associated with intra-epidemic increasing severity in a unique epidemiological setting. Here, we investigated the intra-host genetic diversity in acute human samples collected at different time points during the DENV-3 epidemic occurred in Cuba, 2001-2002 using a deep sequencing approach. We concluded that greater variability in significant minor populations occurred as the epidemic progressed, particularly in the non-structural genes, with higher variability observed in secondary infected cases. Remarkably, for the first time significant intra-host genetic variation was demonstrated within the same patient during the course of secondary infection DENV-1/DENV-3, including changes in structural proteins. These findings indicate that high-resolution approaches are needed to unravel molecular mechanisms involved in dengue pathogenesis.

Molecular Characterization and Risk Factors of Giardia duodenalis among School Children from La Habana, Cuba.
Jerez Puebla LE, Núñez FA, Martínez Silva I, Rojas Rivero L, Martínez González M, Méndez Sutil Y, et al. J Parasitol Res. 2015;2015:378643. DOI: 10.1155/2015/378643. Epub 2015 Nov 29.

Giardia duodenalis is considered the most common protozoan infecting humans worldwide. Molecular characterization of G. duodenalis isolates has revealed the existence of eight groups (assemblages A to H) which differ in their host distribution. A cross-sectional study was conducted in 639 children from La Habana between January and December 2013. Two assemblage-specific PCRs were carried out for the molecular characterization. The overall prevalence of Giardia infection was 11.9%. DNA from 63 of 76 (82.9%) samples was successfully amplified by PCR-tpi, while 58 from 76 (76.3%) were detected by PCRE1-HF. Similar results by both PCRs were obtained in 54 from 76 samples (71%). According to these analyses, assemblage B and mixed assemblages A + B account for most of the Giardia infections in the cohort of children tested. Our current study identified assemblage B as predominant genotype in children infected with Giardia. Univariate

analysis indicated that omission of washing hands before eating and keeping dogs at home were significant risk factors for a Giardia infection. In the future, novel molecular tools for a better discrimination of assemblages at the sub assemblages level are needed to verify possible correlations between Giardia genotypes and symptomatology of giardiasis.

Multicenter study highlighting clinical relevance of new high-throughput methodologies in molecular epidemiology of *Pneumocystis jirovecii* pneumonia.
Esteves F, de Sousa B, Calderón EJ, Huang L, Badura R, Maltez F, et al. Clin Microbiol Infect. 2016 Mar 25. DOI: 10.1016/j.cmi.2016.03.013. [Epub ahead of print]

Pneumocystisjirovecii causes severe interstitial pneumonia (PcP) in immunosuppressed patients. This multicenter study assessed the distribution frequencies of epidemiologically relevant genetic markers of P. jirovecii in different geographic populations from Portugal, the USA, Spain, Cuba and Mozambique, and the relationship between the molecular data and the geographical and clinical information, based on a multifactorial approach. The high-throughput typing strategy for P. jirovecii characterization consisted of DNA pooling using quantitative real-time PCR (qPCR) followed by multiplex-PCR/single base extension (MPCR/SBE). The frequencies of relevant P. jirovecii single nucleotide polymorphisms (SNP) (mt85, SOD110, SOD215, DHFR312, DHPS165 and DHPS171) encoded at four loci were estimated in 10 DNA pooled samples representing a total of 182 individual samples. Putative multilocus genotypes (MLG) of P. jirovecii showed to be clustered due to the geographic differences but also dependent on clinical characteristics of the populations studied. The haplotype DHFR312T/SOD110C/SOD215T was associated with severe AIDS-related PcP and high P. jirovecii burdens. The frequencies of this genetic variant of P. jirovecii were significantly higher in patients with AIDS-related PcP from Portugal and the USA than in the colonized patients from Portugal, Spain, Cuba or Mozambique, highlighting the importance of this haplotype, apparently associated with the severity of the disease. Patients from the USA and Mozambique showed higher rates of DHPS mutants, which may suggest the circulation of P. jirovecii organisms potentially related with TMP-SMX resistance in those geographical regions. This report assessed the worldwide distribution of P. jirovecii haplotypes and their epidemiological impact in distinct geographic and clinical populations.

Needle-free jet injector intradermal delivery of fractional dose inactivated poliovirus vaccine: Association between injection quality and immunogenicity.
Resik S, Tejeda A, Mach O, Sein C, Molodecky N, Jarrahian C, et al.
Vaccine. 2015 Oct 26;33(43):5873–7.

Introduction The World Health Organization recommends that as part of the polio end-game strategy a dose of inactivated poliovirus vaccine (IPV) be introduced by the end of 2015 in all countries currently using only oral poliovirus vaccine (OPV). Administration of fractional dose (1/5 of full dose) IPV (fIPV) by intradermal (ID) injection may reduce costs, but its conventional administration is with Bacillus Calmette-Guerin (BCG) needle and syringe (NS), which is time consuming and technically challenging. We compared injection quality achieved with BCG NS and three needle-free jet injectors and assessed ergonomic features of the injectors. **Methods** Children between 12 and 20 months of age who had previously received OPV were enrolled in the Camaguey, Cuba study. Subjects received a single fIPV dose administered intradermally with BCG NS or one of three needle-free injector devices: BiojectBiojector 2000(®) (B2000), Bioject ID Pen(®) (ID Pen), or PharmaJetTropis(®) (Tropis). We measured bleb diameter and vaccine loss as indicators of ID injection quality, with desirable injection quality defined as bleb diameter ≥5mm and vaccine loss <10%. We surveyed vaccinators to evaluate ergonomic features of the injectors. We further assessed the injection quality indicators as predictors of immune response, measured by increase in poliovirus neutralizing antibodies in blood between day 0 (pre-IPV) and 21 (post-vaccination). **Results** Delivery by BCG NS and Tropis resulted in the highest proportion of subjects with desirable injection quality; health workers ranked Biojector2000 and Tropis highest for ergonomic features. We observed that vaccine loss and desirable injection quality were associated with an immune response for poliovirus type 2 (P=0.02, P=0.01, respectively). **Conclusions**Our study demonstrated the feasibility of fIPV delivery using needle-free injector devices with high acceptability among health workers. We did not observe the indicators of injection quality to be uniformly associated with immune response.

Protective T cell and antibody immune response against Hepatitis C Virus using the biopolyester beads based vaccine delivery system.
Martínez-Donato G, Piniella B, Aguilar D, Olivera S, Pérez A, Castañedo Y, et al. Clin Vaccine Immunol. 2016 Feb 17.pii: CVI.00687-15. [Epub ahead of print]

Hepatitis C virus (HCV) infection is a major worldwide problem. Chronic hepatitis C is recognized as one of the major causes of cirrhosis, hepatocellular carcinoma and liver failure. Although, new directly acting antiviral therapies are suggested to overcome the low efficacy and adverse effects observed for the current standard of treatment, an effective vaccine would be the only way to certainly eradicate HCV-infection. Recently, polyhydroxybutyrate beads produced by engineered *Escherichia coli* showed efficacy as vaccine delivery system. Here, an endotoxin-free *E. coli* strain (ClearColi) was engineered to produce polyhydroxybutyrate beads displaying the core antigen on their surface (Beads-Core) and their immunogenicity was evaluated in BALB/c mice. Immunization with Beads-Core induced IFN-γ secretion and a functional T cell immune response against the HCV core protein. With the aim to target broad T and B cell determinants described for HCV, Beads-Core mixed with HCV E1, E2 and NS3 recombinant proteins was also evaluated in BALB/c mice. Remarkably, only three immunization with Beads-Core+E1E2NS3/Alum induced a potent antibody response against E1, E2 and a broad IFN-γ secretion and T cell response, against Core and all co-administered antigens. This immunological response mediated protective immunity against viremia as assessed in a viral surrogate challenge model. Overall it was shown that engineered biopolyester beads displaying foreign antigens are immunogenic and might present a particulate delivery system suitable for vaccination against HCV.

Relevance of Non-communicable Comorbidities for the Development of the Severe Forms of Dengue: A Systematic Literature Review.
Toledo J, George L, Martínez E, Lázaro A, Han WW, Coelho GE, et al. PLoS Negl Trop Dis. 2016 Jan 4;10(1):e0004284. DOI: 10.1371/journal.pntd.0004284.

Patients with dengue fever and comorbidities seem to be at higher risk of developing complications and/or severe dengue compared to healthier individuals. This study systematically reviews the evidence related to comorbidities and dengue. A systematic literature review was performed in five databases (EMBASE, PUBMED, Global Health, SciELO, Cochrane) and grey literature for full-text articles since its inceptions until October 10, 2015. A total of 230 articles were retrieved. Sixteen studies were analysed after applying all inclusion and exclusion criteria. Seven case control studies and nine retrospective cohort studies showed that comorbidities may contribute to severe dengue, especially 1) cardiovascular disease, 2) stroke, 3) diabetes, 4) respiratory disease and 5) renal disease, as well as old age. However, due to heterogeneity in studies, the real estimate effect of comorbidities as modifiers of dengue severity could not be established. Further research in regions with high prevalence of dengue infection would contribute to a better understanding of the relevance of comorbidities in severe dengue, especially with a standardised protocol, for outcomes, specific comorbidities, study design—best using prospective designs—and sample sizes.

Renal Cancer in the Elderly.
González León T, Morera Pérez M. Curr Urol Rep. 2016 Jan;17(1):6. DOI: 10.1007/s11934-015-0562-2.

The increase of the aging population corresponds with the rise of renal cancer in elderly patients. The distinction between functional and chronological age, quality of life, and survival estimate are important issues, among others, that should be considered in the management of renal cancer in elderly patients. We made this review with the purpose of synthesizing the most updated criteria regarding indications and outcomes of the different therapeutic options in the management of elderly patients with renal cancer, beginning from the physiologic considerations that characterize them, their capacity to tolerate different therapeutic possibilities, and the prognosis of the patients' risks and comorbidity assessment.

Serum neuron specific enolase could predict subclinical brain damage and the subsequent occurrence of brain related vascular events during follow up in essential hypertension.
González-Quevedo A, González-García S, Hernández-Díaz Z, Fernández Concepción O, Quevedo Sotolongo L, Peña-Sánchez M. J Neurol Sci. 2016 Apr 15;363:158–63.

The object of this work was to explore if blood based biomarkers of brain damage could predict subclinical brain lesions and clinical outcome during follow-up in asymptomatic hypertensive patients. This was a cross-sectional study including 101 patients with essential hypertension and no clinical evidence of neurological disease and 53 healthy controls, followed by a longitudinal study of 62 hypertensive patients for an average of 33months. Serum concentrations of two brain specific proteins (S100B and neuron specific enolase - NSE) were determined at inclusion. Fundoscopic exploration, brain MRI and echocardiographic studies were also performed. Clinical outcome at follow-up was registered: transient ischemic attack (TIA), stroke, vascular headache or migraine, cardiovascular events and death. Higher serum NSE and S100B concentrations were observed in hypertensive patients; and multiple regression analysis revealed independent associations of clinical variables and more severe whitematter lesions only with NSE concentration. A panel combining two

clinical variables (blood pressure>140/90 and years of hypertension>10) and serum NSE>13μg/L predicted more severe white matter lesions with 80% sensitivity and 94.4% specificity. Higher NSE levels at inclusion were associated not only with the occurrence of vascular events related with the CNS (stroke, TIA and vascular headache), but also with an earlier presentation of these events during the follow-up period. Serum NSE concentration could be a useful biomarker to predict subclinical brain damage and future vascular events related with the CNS in hypertension. Blood based biomarkers could aid in filtering hypertensive patients with a higher risk of cerebrovascular disease for brain MRI scanning.

Simple Enucleation for Renal Tumors: Indications, Techniques, and Results.
García AG, León TG. Curr Urol Rep. 2016 Jan;17(1):7.

Kidney cancer is the 13th most common malignancy worldwide with significant increase in Stage I renal cell cancer (RCC). Surgical excision by nephron sparing surgery (NSS) remains the treatment of choice for small renal masses (SRMs). One of the variants of partial nephrectomy is simple enucleation (SE) or tumor enucleation (TE). The present review comments on the indications and technical aspects of SE as well as its outcomes. SE/TE has shown both perioperative and oncologic satisfactory results, comparable to partial nephrectomy (PN). It is a valid technique for SRMs and achieves maximum renal parenchymal preservation with an insignificant impact on renal function.

Spinocerebellar ataxia type 2: Measures of saccade changes improve power for clinical trials.
Rodríguez-Labrada R, Velázquez-Pérez L, Auburger G, Ziemann U, Canales-Ochoa N, Medrano-Montero J, et al. Mov Disord. 2016 Feb 5. DOI: 10.1002/mds.26532. [Epub ahead of print]

Background Saccadic eye movement abnormalities are common in patients with spinocerebellarataxiatype2, but it is unclear how these alterations progress over time. The aim of this study was to assess the progression of saccade involvement in spinocerebellarataxiatype2 patients, identify its main determinants, and evaluate its usefulness as outcome measures in clinical trials.**Methods** A prospective 5-year follow-up study was performed with 30 spinocerebellarataxiatype2 patients and their matched healthy controls, who were evaluated a total of four times by clinical and electrooculographical assessments of horizontal saccades and by the scoring of ataxia. **Results**Patients showed significant decreases in saccade peak velocity and saccade accuracy as well as increases of saccadic latency during the follow-up period. Annual progression rates were significantly higher in patients compared to controls. Faster progression rates of saccade slowing were associated with higher trinucleotide cytosine-adenine-guanine repeat expansions. Sample-size estimates for two-arm trials would require 19 patients per group to detect a 50% reduction in disease progression using saccade peak velocity as outcome variable, but 44 and 124 patients using saccade latency and accuracy, respectively (power, 80%; alpha<0.05). **Conclusions** Electrooculographical measures of saccade changes are useful for the objective quantification of disease course in spinocerebellar ataxia type2. The progression rate of saccade slowing is influenced by the expansion size, providing novel insight into the cumulative polyglutamine neurotoxicity, and supporting the usefulness of saccade peak velocity as a sensitive biomarker during the natural history of the disease, and as suitable outcome measure for therapeutic trials.

Superior Efficacy and Safety of a Nonemulsive Variant of the NGcGM3/VSSP Vaccine in Advanced Breast Cancer Patients.
de la Torre A, Pérez K, Vega AM, Santiesteban E, Ruiz R, Hernández L, et al. Breast Cancer (Auckl). 2016 Feb 18;10:5–11. DOI: 10.4137/BCBCR.S32785.

NGcGM3 ganglio side is a tumor-specific antigen expressed in human breast tumors. The NGcGM3/VSSP vaccine, consisting in very small-sized proteoliposomes (VSSP) obtained by the incorporation of NGcGM3 into the outer membrane protein complex of Neisseria meningitidis, has been previously tested in a Phase II trial in patients with metastatic breast cancer (MBC) but emulsified with Montanide ISA 51. An Expanded Access study was carried out in MBC patients aiming to find if a nonemulsive formulation of NGcGM3/VSSP, without Montanide ISA 51, could be more safe and effective. A total of 104 patients were vaccinated with the nonemulsive formulation (900 μg), subcutaneously (SC), or with the emulsive formulation (200 μg), intramuscularly (IM). An intent-to-treat analysis of efficacy was performed with all patients, and 93 patients were split off according to the site of metastases (visceral/nonvisceral). Of note, SC-treated patients exhibited a superior median overall survival (OS) than IM-treated patients (23.6 vs. 8.2 months; log rank P = 0.001). Even though in the subset of patients with nonvisceral metastases SC vaccination duplicated the median OS compared to the alternative option (31.6 vs. 16.5 months), this difference did not reach statistical significance (log rank P = 0.118). Curiously, in patients with visceral metastases, the advantage of the nonemulsive

formulation was more apparent (median OS 21.0 vs. 6.2 months; log rank P = 0.005). The vaccine was safe for both formulations.

The number of optic neuritis attacks is a potential confounder when comparing patients with NMO vs. controls by voxel-based neuroimaging analysis.
Sánchez-Catasús CA,Cabrera-Gómez J, Almaguer Melián W, Bosch Bayard J, Rodríguez Rojas R, Valdés-Sosa P. Acta Radiol.2015 Oct 25.pii: 0284185115610935. [Epub ahead of print]

Background Voxel-based morphometric (VBM) studies in neuromyelitis optica (NMO) have shown limited reproducibility. A previous study suggests that the number of optic neuritis (ON) attacks may be a confounding factor when comparing NMO patients with controls if it is not taken into account during VBM analysis. **Purpose** To investigate the potential confounding effect of the number of ON attacks, for both tissue volumes and perfusion by voxel-based statistical analysis. **Material and methods** Volumetric magnetic resonance imaging (MRI) and perfusion SPECT were obtained from 15 controls and two patient subgroups: subgroup I was composed of nine patients with one or two ON attacks; and subgroup II of six patients with three or four ON attacks. We performed non-parametric voxel-based comparison of tissue volumes and perfusion between controls versus the two patient subgroups and for the whole patient group. **Results** Subgroup I presented no volume reductions, contrary to subgroup II that showed unequivocal reduction. We also found hypoperfusion in different brain regions in different subgroups. The results were quite different for the whole patient group. **Conclusion** These findings highlight the confounding effect of the number of ON attacks, providing a new methodological insight that could explain the limited reproducibility of previous VBM studies in NMO.

Validating Non-invasive EEG Source Imaging Using Optimal Electrode Configurations on a Representative Rat Head Model.
Valdés-Hernández PA, Bae J, Song Y, Sumiyoshi A, Aubert-Vázquez E, Riera JJ. Brain Topogr. 2016 Mar 30. [Epub ahead of print]

The curtain of technical limitations impeding rat multichannel non-invasive electroencephalography (EEG) has risen. Given the importance of this preclinical model, development and validation of EEGsourceimaging (ESI) is essential. We investigate the validity of well-known human ESI methodologies in rats which individual tissue geometries have been approximated by those extracted from an MRI template, leading also to imprecision in electrode localizations. With the half and fifth sensitivity volumes we determine both the theoretical minimum electrode separation for non-redundant scalp EEG measurements and the electrode sensitivity resolution, which vary over the scalp because of the head geometry. According to our results, electrodes should be at least ~3 to 3.5 mm apart for an optimal configuration. The sensitivity resolution is generally worse for electrodes at the boundaries of the scalp measured region, though, by analogy with human montages, concentrates the sensitivity enough to localize sources. Cramér-Rao lower bounds of source localization errors indicate it is theoretically possible to achieve ESI accuracy at the level of anatomical structures, such as the stimulus-specific somatosensory areas, using the template. More validation for this approximation is provided through the comparison between the template and the individual lead field matrices, for several rats. Finally, using well-accepted inverse methods, we demonstrate that somatosensory ESI is not only expected but also allows exploring unknown phenomena related to global sensory integration. Inheriting the advantages and pitfalls of human ESI, rat ESI will boost the understanding of brain pathophysiological mechanisms and the evaluation of ESI methodologies, new pharmacological treatments and ESI-based biomarkers.