

## Cuban Cancer Research in International Journals

The following selection—alphabetical by title—reflects Cuban publishing on cancer in international medical and population health journals from January 2011 through July 2014.

**[Absolute lymphocyte count as a prognostic factor in children with acute lymphoblastic leukemia]**. Anoceto Martínez A, González Otero A, Guerchicoff de Svarch E, Arencibia Nuñez A, Jaime JC, Dorticos E, et al. *An Pediatr (Barc)*. 2012 Jan;76(1):10.e1–6. Spanish.

**Introduction** Recent studies have suggested that the absolute lymphocyte count (ALC) may be a prognostic indicator in malignant diseases, in that those patients who have higher ALC at certain times during treatment may have a better chance of survival. The influence of T cells and natural killer cells in the immune system of the patient with cancer as a response to cancer cells is particularly noted. **Materials and method** We prospectively assessed the prognostic value of absolute lymphocytic count (ALC) in 105 pediatric patients with acute lymphoblastic leukemia (ALL), treated in the Cuban Immunology and Hematology Institute from 1995 to 2008. ALC was studied at days 15 (ALC-15) and 28 (ALC-28) of treatment. **Results** In our patients, 1000 cells/uL was the median ALC value for patients who relapsed or died. Using 1000/uL we found that ALL patients with an ALC-15 <1000 cells/μl had a 5-year relapse free survival (RFS) of 51%. In contrast, an ALC-15 >1000 cells/uL showed an excellent prognosis, with a 5-year RFS of 83% (p=0.02). Similarly in our study, an ALC-28 <1000 cells/μl predicted a 5-year overall survival (OS) of 66%, whereas an ALC-28 >1000 cells/μl predicted excellent outcome, with a 5-year OS of 86% (p=0.01). Importantly, ALC is also a strong predictor in multivariate analysis with known prognostic factors. ALC is a simple, statistically powerful measurement for patients with de novo ALL. **Conclusions** The results, when combined with previous studies, demonstrate that ALC is a powerful new prognostic factor for a range of malignancies.

**A cytotoxic humanized anti-ganglioside antibody produced in a murine cell line defective of N-glycolylated-glycoconjugates**. Fernández-Marrero Y, Roque-Navarro L, Hernández T, Dorvignit D, Molina-Pérez M, González A, et al. *Immunobiology*. 2011 Dec;216(12):1239–47.

Gangliosides containing the N-glycolyl (NGc) form of sialic acid are tumor-associated antigens and promising candidates for cancer therapy. We previously generated the murine 14F7 monoclonal antibody (mAb), specific for the N-glycolyl-GM3 ganglioside (NGcGM3), which induced an oncosis-like type of cell death on malignant cell lines expressing this antigen and recognized breast carcinoma by immunoscintigraphy in cancer patients. As humanization is expected to enhance its use for human cancer therapy, herein we describe the design and generation of two humanized versions of the 14F7 mAb by disrupting potential human T cell epitopes on its variable region. No differences in antigen reactivity or cytotoxic properties were detected among the variants tested and with respect to the chimeric counterpart. Humanized 14F7 genes were transfected into the NGcGM3-expressing NS0 cell line. Therefore, in the industrial scaling-up of the transfectoma in serum-free medium, cell viability was lost due to the cytotoxic effect of the secreted antibody. This shortcoming was solved by knocking down the CMP-N-acetylneuraminic acid hydroxylase enzyme, thus impairing the synthesis of NGc-glycoconjugates. Humanized 14F7 mAb is of potential value for the therapy of NGcGM3-expressing tumors.

**Affinity maturation and fine functional mapping of an antibody fragment against a novel neutralizing epitope on human vascular endothelial growth factor**. Lamdan H, Gavilondo JV, Muñoz Y, Pupo A, Huerta V, Musacchio A, et al. *Mol Biosyst*. 2013 Aug;9(8):2097–106.

We have previously reported the isolation of a novel single-chain variable fragment (scFv) against vascular endothelial growth factor (VEGF), from a phage-displayed human antibody repertoire. This scFv, denominated 2H1, was shown to block the binding of VEGF to its receptor but exhibited a moderate binding affinity. Here, we describe the affinity maturation of the 2H1 scFv. Two phage-displayed libraries were constructed by diversification of the third complementarity-determining regions (CDRs) of the light (VL) and heavy (VH) chain variable domains of 2H1 using parsimonious mutagenesis. A competitive phage-selection strategy in the presence of the parental scFv as a competitor was used to eliminate low affinity binders. High affinity variants were retrieved from both libraries. An optimized VL variant was designed and constructed by combining recurrent replacements found among selected variants in a single molecule, resulting in an additional affinity increase. Further affinity improvements were achieved by combining this optimized VL with the best VH variants. The final variant obtained here, L3H6, showed an overall affinity improvement of 18-fold over the parental scFv and exhibited an enhanced potency to block the binding of VEGF to its receptor. Using phage

display and extensive mutagenesis of VEGF, we determined the fine specificity of L3H6. This functional mapping revealed a novel neutralizing epitope on human VEGF defined by the residues Y25, T71, E72, N100, K101, E103 and R105. The conformational epitope recognized by L3H6 was recapitulated by grafting human VEGF residues into the mouse molecule, providing further confirmation of the nature of the identified epitope.

**Analytical and numerical solutions of the potential and electric field generated by different electrode arrays in a tumor tissue under electrotherapy.** Bergues Pupo AE, Reyes JB, Bergues Cabrales LE, Bergues Cabrales JM. *Biomed Eng Online.* 2011 Sep 24;10:85. doi: 10.1186/1475-925X-10-85.

**Background** Electrotherapy is a relatively well established and efficient method of tumor treatment. In this paper we focus on analytical and numerical calculations of the potential and electric field distributions inside a tumor tissue in a two-dimensional model (2D-model) generated by means of electrode arrays with shapes of different conic sections (ellipse, parabola and hyperbola). **Methods** Analytical calculations of the potential and electric field distributions based on 2D-models for different electrode arrays are performed by solving the Laplace equation, meanwhile the numerical solution is solved by means of finite element method in two dimensions. **Results** Both analytical and numerical solutions reveal significant differences between the electric field distributions generated by electrode arrays with shapes of circle and different conic sections (elliptic, parabolic and hyperbolic). Electrode arrays with circular, elliptical and hyperbolic shapes have the advantage of concentrating the electric field lines in the tumor. **Conclusion** The mathematical approach presented in this study provides a useful tool for the design of electrode arrays with different shapes of conic sections by means of the use of the unifying principle. At the same time, we verify the good correspondence between the analytical and numerical solutions for the potential and electric field distributions generated by the electrode array with different conic sections.

**Anti-ganglioside anti-idiotypic vaccination: more than molecular mimicry.** Vázquez AM, Rodríguez-Zhurbenko N, López AM. *Front Oncol.* 2012 Nov 20;2:170. doi: 10.3389/fonc.2012.00170. eCollection 2012.

Surgery, chemotherapy, and radiation therapy are standard modalities for cancer treatment, but the effectiveness of these treatments has reached a plateau. Thus, other strategies are being explored to combine with the current treatment paradigms in order to reach better clinical results. One of these approaches is the active immunotherapy based on the induction of anti-tumor responses by anti-idiotypic vaccination. This approach arose from Jerne's idiotypic network theory, which postulates that B lymphocytes forms a functional network, with a role in the establishment of the immune repertoires, in the regulation of natural antibody production and even in the establishment of natural tolerance. Due to the large potential diversity of the immunoglobulin variable regions, the idiotypes repertoire can mimic the universe of self and foreign epitopes, even those of non-protein nature, like gangliosides. Gangliosides are sialic acid-containing glycolipids that have been considered attractive targets for cancer immunotherapy, based on the qualitative and quantitative changes they suffer during malignant transformation and due to their importance for tumor biology. Although any idioype could be able to mimic any antigen, only those related to antigens involved in functions relevant for organism homeostasis, and that in consequence has been fixed by evolution, would be able not only to mimic, but also to activate the idiotypic cascades related with the nominal antigen. The present review updates the results, failures and hopes, obtained with ganglioside mimicking anti-idiotypic antibodies and presents evidences of the existence of a natural response against gangliosides, suggesting that these glycolipids could be idiotypically relevant antigens.

**Antigen dose escalation study of a VEGF-based therapeutic cancer vaccine in non human primates.** Morera Y, Bequet-Romero M, Ayala M, Pérez PP, Castro J, Sánchez J, et al. *Vaccine.* 2012 Jan 5;30(2):368–77.

CIGB-247 is a cancer therapeutic, based on recombinant modified human vascular endothelial growth factor (VEGF) as antigen, in combination with the oil free adjuvant VSSP (very small sized proteoliposomes of *Neisseria meningitidis* outer membrane). Our previous experimental studies in mice with CIGB-247 have shown that the vaccine has both anti-tumoral and anti-metastatic activity, and produces both antibodies that block VEGF-VEGF receptor interaction, and a specific T-cell cytotoxic response against tumor cells. CIGB-247, with an antigen dose of 100 µg, has been characterized by an excellent safety profile in mice, rats, rabbits, and non human primates. In this article we extend the immunogenicity and safety studies of CIGB-247 in non human primates, scaling the antigen dose from 100 µg to 200 and 400 µg/vaccination. Our results indicate that such dose escalation did not affect animal behavior, clinical status, and blood parameters and biochemistry. Also, vaccination did not interfere with skin deep skin wound healing. Anti-VEGF IgG antibodies and specific T-cell mediated responses were documented at all three studied doses. Antigen dose apparently did not

determine differences in maximum antibody titer during the 8 weekly immunization induction phase, or the subsequent increase in antibodies seen for monthly boosters delivered afterwards. Higher antigen doses had a positive influence in antibody titer maintenance, after cessation of immunizations. Boosters were important to achieve maximum antibody VEGF blocking activity, and specific T-cell responses in all individuals. Purified IgG from CIGB-247 immunized monkey sera was able to impair proliferation and formation of capillary-like structures in Matrigel, for HMEC cells in culture. Altogether, these results support the further clinical development of the CIGB-247 therapeutic cancer vaccine, and inform on the potential mechanisms involved in its effect.

**Anti-NeuGcGM3 antibodies, actively elicited by idiotypic vaccination in nonsmall cell lung cancer patients, induce tumor cell death by an oncosis-like mechanism.** Hernández AM, Rodríguez N, González JE, Reyes E, Rondón T, Griñán T, et al. *J Immunol.* 2011 Mar 15;186(6):3735–44.

1E10 is a murine anti-idiotypic mAb specific for an idiotypic mAb that reacts with NeuGc-containing gangliosides, sulfatides, and Ags expressed in some human tumors. In melanoma, breast, and lung cancer patients, this anti-idiotypic Ab was able to induce a specific Ab response against N-glycosylated gangliosides, attractive targets for cancer immunotherapy as these glycolipids are not naturally expressed in humans. A clinical study with nonsmall cell lung cancer patients showed encouraging clinical benefits. Immunological studies performed in 20 of these patients suggested a correlation between the induction of Abs against NeuGcGM3 and longer survival times. The induced anti-NeuGcGM3 Abs recognized and directly killed tumor cells expressing the Ag, by a mechanism independent of complement activation. In the present work, we show that this cytotoxicity differs from apoptosis because it is temperature independent, no chromatin condensation or caspase 3 induction are detected, and the DNA fragmentation induced has a different pattern than the one characteristic for apoptosis. It is a very quick process and involves cytoskeleton reorganization. The Abs induce cellular swelling and the formation of big membrane lesions that allow the leakage of cytoplasm and the loss of the cell membrane integrity. All of these characteristics resemble a process of oncotic necrosis. To our knowledge, this is the first report of the active induction in cancer patients of NeuGcGM3-specific Abs able to induce complement independent oncotic necrosis to tumor cells. These results contribute to reinforcing the therapeutic potential of anti-idiotypic vaccines and the importance of NeuGcGM3 ganglioside as antitumor target.

**Antitumor effects of electrochemical treatment.** Ciria HM, González MM, Zamora LO, Cabrales LE, Sierra González GV, de Oliveira LO, et al. *Chin J Cancer Res.* 2013 Apr;25(2):223–34.

Electrochemical treatment is an alternative modality for tumor treatment based on the application of a low intensity direct electric current to the tumor tissue through two or more platinum electrodes placed within the tumor zone or in the surrounding areas. This treatment is noted for its great effectiveness, minimal invasiveness and local effect. Several studies have been conducted worldwide to evaluate the antitumoral effect of this therapy. In all these studies a variety of biochemical and physiological responses of tumors to the applied treatment have been obtained. By this reason, researchers have suggested various mechanisms to explain how direct electric current destroys tumor cells. Although, it is generally accepted this treatment induces electrolysis, electroosmosis and electroporation in tumoral tissues. However, action mechanism of this alternative modality on the tumor tissue is not well understood. Although the principle of Electrochemical treatment is simple, a standardized method is not yet available. The mechanism by which Electrochemical treatment affects tumor growth and survival may represent more complex process. The present work analyzes the latest and most important research done on the electrochemical treatment of tumors. We conclude with our point of view about the destruction mechanism features of this alternative therapy. Also, we suggest some mechanisms and strategies from the thermodynamic point of view for this therapy. In the area of Electrochemical treatment of cancer this tool has been exploited very little and much work remains to be done. Electrochemical treatment constitutes a good therapeutic option for patients that have failed the conventional oncology methods.

**Antitumor efficacy, pharmacokinetic and biodistribution studies of the anticancer peptide CIGB-552 in mouse models.** Vallespí MG, Pimentel G, Cabrales-Rico A, Garza J, Oliva B, Mendoza O, et al. *J Pept Sci.* 2014 Jul 20. [Epub ahead of print]

Accumulation of the COMMD1 protein as a druggable pharmacology event to target cancer cells has not been evaluated so far in cancer animal models. We have previously demonstrated that a second-generation peptide, with cell-penetrating capacity, termed CIGB-552, was able to induce apoptosis mediated by stabilization of COMMD1. Here, we explore the antitumor effect by subcutaneous administration of CIGB-552 in a therapeutic

schedule. Outstandingly, a significant delay of tumor growth was observed at 0.2 and 0.7 mg/kg ( $p < 0.01$ ) or 1.4 mg/kg ( $p < 0.001$ ) after CIGB-552 administration in both syngeneic murine tumors and patient-derived xenograft models. Furthermore, we evidenced that 131 I-CIGB-552 peptide was actually accumulated in the tumors after administration by subcutaneous route. A typical serine-proteases degradation pattern for CIGB-552 in BALB/c mice serum was identified. Further, biological characterization of the main metabolites of the peptide CIGB-552 suggests that the cell-penetrating capacity plays an important role in the cytotoxic activity. This report is the first in describing the antitumor effect induced by systemic administration of a peptide that targets COMMD1 for stabilization. Moreover, our data reinforce the perspectives of CIGB-552 for cancer targeted therapy.

**Application of hazard models for patients with breast cancer in Cuba.** Alfonso AG, de Oca NA. *Int J Clin Exp Med.* 2011;4(2):148–56.

There has been a rapid development in hazard models and survival analysis in the last decade. This article aims to assess the overall survival time of breast cancer in Cuba, as well as to determine plausible factors that may have a significant impact in the survival time. The data are obtained from the National Cancer Register of Cuba. The data set used in this study relates to 6381 patients diagnosed with breast cancer between January 2000 and December 2002. Follow-up data are available until the end of December 2007, by which time 2167 (33.9%) had died and 4214 (66.1%) were still alive. The adequacy of six parametric models is assessed by using their Akaike information criterion values. Five of the six parametric models (Exponential, Weibull, Log-logistic, Lognormal, and Generalized Gamma) are parameterized by using the accelerated failure-time metric, and the Gompertz model is parameterized by using the proportional hazard metric. The main result in terms of survival is found for the different categories of the clinical stage covariate. The survival time among patients who have been diagnosed at early stage of breast cancer is about 60% higher than the one among patients diagnosed at more advanced stage of the disease. Differences among provinces have not been found. The age is another significant factor, but there is no important difference between patient ages.

**A shift from N-glycolyl- to N-acetyl-sialic acid in the GM3 ganglioside impairs tumor development in mouse lymphocytic leukemia cells.** Casadesús AV, Fernández-Marrero Y, Clavell M, Gómez JA, Hernández T, Moreno E, et al. *Glycoconj J.* 2013 Oct;30(7):687–99.

Humans, in contrast to other mammals, do not synthesize N-glycolyl-neuraminic acid (Neu5Gc) due to a deletion in the gene (*cmah*) encoding the enzyme responsible for this conversion, the cytidine monophospho-N-acetyl-neuraminic acid hydroxylase (CMP-Neu5Ac hydroxylase). The detection of considerable amounts of Neu5Gc-sialoconjugates, in particular gangliosides, in human malignancies makes these antigens attractive targets for immunotherapy, in particular with monoclonal antibodies (mAbs). We have previously described a GM3(Neu5Gc) ganglioside-specific mAb, named 14F7, with the ability to kill tumor cells in a complement-independent manner. Silencing the *cmah* gene in GM3(Neu5Gc)-expressing L1210 mouse lymphocytic leukemia B cells caused the abrogation of this cytotoxic effect. We now show that *cmah*-silenced L1210 cells (*cmah*-kd) express a high level of GM3(Neu5Ac) and have an impaired ability for anchorage-independent cell growth and tumor development *in vivo*. No evidences of increased immunogenicity of the *cmah*-kd cell line were found. These results provide new evidences on the role of GM3(Neu5Gc), or Neu5Gc-sialoconjugates in general, in tumor biology. As an important tool in this study, we used the humanized version (here referred to as 7C1 mAb) of a recently described, rationally-designed mutant of 14F7 mAb that is able to bind to both GM3(Neu5Gc) and GM3(Neu5Ac). In contrast to its parental antibody, the humanized 14F7 (14F7hT) mAb, 7C1 mAb was able to kill not only GM3(Neu5Gc)-expressing L1210 wild type cells, but also GM3(Neu5Ac)-expressing *cmah*-kd cells, which endorses this antibody as a potential agent for cancer immunotherapy.

**Assessment of infection with polyomaviruses BKV, JCV and SV40 in different groups of Cuban individuals.** Martínez PA, Kourí V, Cordero G, Correa C, Soto Y, Capó V, et al. *Arch Virol.* 2012 Feb;157(2):315–21.

We investigated the frequency of BKV, JCV and SV40 reactivation in three groups of Cuban patients by multiplex nested PCR assay of 40 paraffin-embedded colorectal neoplasm tissues, 113 urine samples, and 125 plasma samples from 27 transplant recipients, and cerebrospinal fluid (CSF) from 67 HIV-1-infected individuals with central nervous system (CNS) disorders. None of these polyomaviruses were detected in colorectal neoplasms. JCV DNA was detected in 2 of 67 patients (2.9%) with CNS disorders, but neither BKV nor SV40 was identified. BKV was found in urine from 38.5% and 28.6% of adult and pediatric transplant recipients, respectively. In adult renal transplant recipients, excretion of BKV in urine was significantly associated with episodes of acute rejection ( $p=0.012$ ) and with excretion of HCMV in urine ( $p=0.008$ ). In Cuba,

the polyomaviruses studied here could not be related to colorectal neoplasms, and JCV was rarely detected in CSFs of HIV-1-infected individuals, whilst BKV reactivation was found to occur frequently in organ transplant recipients.

[A view on EGFR-targeted therapies from the oncogene-addiction perspective.](#) Perez R, Crombet T, de Leon J, Moreno E. *Front Pharmacol.* 2013 Apr 26;4:53. doi:10.3389/fphar.2013.00053. eCollection 2013.

Tumor cell growth and survival can often be impaired by inactivating a single oncogene- a phenomenon that has been called as "oncogene addiction." It is in such scenarios that molecular targeted therapies may succeed. among known oncogenes, the epidermal growth factor receptor (EGFR) has become the target of different cancer therapies. So far, however, the clinical benefit from EGFR-targeted therapies has been rather limited. a critical review of the large amount of clinical data obtained with anti-EGFR agents, carried out from the perspective of the oncogene addiction concept, may help to understand the causes of the unsatisfactory results. In this article we intend to do such an exercise taking as basis for the analysis a few case studies of anti-EGFR agents that are currently in the clinic. There, the "EGFR addiction" phenomenon becomes apparent in high-responder patients. We further discuss how the concept of oncogene addiction needs to be interpreted on the light of emerging experimental evidences and ideas; in particular, that EGFR addiction may reflect the interconnection of several cellular pathways. In this regard we set forth several hypotheses; namely, that requirement of higher glucose uptake by hypoxic tumor cells may reinforce EGFR addiction; and that chronic use of EGFR-targeted antibodies in EGFR-addicted tumors would induce stable disease by reversing the malignant phenotype of cancer stem cells and also by sustaining an anti-tumor T cell response. Finally, we discuss possible reasons for the failure of certain combinatorial therapies involving anti-EGFR agents, arguing that some of these agents might produce either a negative or a positive trans-modulation effect on other oncogenes. It becomes evident that we need operational definitions of EGFR addiction in order to determine which patient populations may benefit from treatment with anti-EGFR drugs, and to improve the design of these therapies.

[Cancer survival in Cuba, 1994-1995.](#) Garrote LF, Alvarez YG, Babie PT, Yi MG, Alvarez MG, Cicili ML. *IARC Sci Publ.* 2011;(162):89-95.

The population-based cancer registry in Cuba is a national cancer registry established in 1964; cancer registration is entirely done by passive methods. Data on survival from 13 cancer sites or types registered during 1994-1995 are reported. Follow-up has been carried out predominantly by passive methods, with median follow-up ranging from 13-54 months. The proportion with histologically verified diagnosis for various cancers ranged between 34-100%; death certificates only (DCOs) comprised 8-50%; 50-89% of total registered cases were included for the survival analysis. The 5-year age-standardized relative survival for selected cancers were breast (69%), colon (41%), cervix (56%), urinary bladder (64%), rectum (48%) and non-Hodgkin lymphoma (49%). The 5-year relative survival by age group showed no distinct pattern or trend, and was fluctuating. A decreasing survival with increasing clinical extent of disease was noted for all cancers studied. The data on survival trend revealed that the 5-year relative survival of most cancers diagnosed in 1994-1995 was greater than that in 1988-1989.

[Cancer vaccine characterization: from bench to clinic.](#) de la Luz-Hernández K, Rabasa Y, Montesinos R, Fuentes D, Santo-Tomás JF, Morales O, et al. *Vaccine.* 2014 May 19;32(24):2851-8.

**Background** The development of safe, effective, and affordable vaccines has become a global effort due to its vast impact on overall world health conditions. A brief overview of vaccine characterization techniques, especially in the area of high-resolution mass spectrometry, is presented. It is highly conceivable that the proper use of advanced technologies such as high-resolution mass spectrometry, along with the appropriate chemical and physical property evaluations, will yield tremendous in-depth scientific understanding for the characterization of vaccines in various stages of vaccine development. This work presents the physicochemical and biological characterization of cancer vaccine Racotumomab/alumina, a murine anti-idiotypic antibody that mimics N-glycolyl-GM3 gangliosides. This antibody has been tested as an anti-idiotypic cancer vaccine, adjuvated in Al(OH)<sub>3</sub>, in several clinical trials for melanoma, breast, and lung cancer. **Methods** Racotumomab was obtained from ascites fluid, transferred to fermentation in stirred tank at 10 L and followed to a scale up to 41 L. The mass spectrometry was used for the determination of intact molecule, light and heavy chains masses; amino acids sequence analysis, N- and C-terminal, glycosylation and posttranslational modifications. Also we used the DLS for the size distribution and zeta potential analysis. The biological analyses were performed in mice and chickens. **Results** We observed differences in glycosylation pattern, charge heterogeneity and structural stability between in vivo-produced and bioreactor-

obtained Racotumomab products. Interestingly, these modifications had no significant impact on the immune responses elicited in two different animal models. **Conclusions** We are demonstrated that this approach could potentially be more efficient and effective for supporting vaccine research and development.

**Challenges and opportunities for cancer vaccines in the current NSCLC clinical scenario.** Rodriguez PC, Sanchez B. *Curr Top Med Chem.* 2013;13(20):2551–61.

This review is aimed to focus on NSCLC as an emerging and promising model for active immunotherapy and the challenges for its inclusion in the current clinical scenario. Cancer vaccines for NSCLC have been focused as a therapeutic option based on the identification of a tumor hallmark and the active immunization with the related molecules that triggers cellular and/or humoral responses that consequently destroy or delay the rate of malignant progression. This therapeutic intervention in an established disease state has been aimed to impact into prolonging patient's survival with ethically accepted quality of life. Understanding of relationship between structure and function in cancer vaccines is essential to interpret their opportunities to impact into prolonging survival and increasing quality of life in cancer patients. It is widely accepted that the failure of the cancer vaccines in the NSCLC scenario is related with its introduction in the advanced disease stages and poor performance status of the patients due to the combination of the tumor induced immunosuppression with the immune senescence. Despite first, second and emerging third line of onco-specific treatments the life expectancy for NSCLC patients diagnosed at advanced stages is surrounding the 12 months of median survival and in facts the today real circumstances are extremely demanding for the success inclusion of cancer vaccines as therapeutic choice in the clinical scenario. The kinetics of the active immunizations encompasses a sequential cascade of clinical endpoints: starting by the activation of the immune system, followed by the antitumor response and finalizing with the consequential impact on patients' overall survival. Today this cascade of clinical endpoints is the backbone for active immunization assessment and moreover the concept of cancer vaccines, applied in the NSCLC setting, is just evolving as a complex therapeutic strategy, in which the opportunities for cancer vaccines start from the selection of the target cancer hallmark, followed by the vaccine formulation and its platforms for immune potentiating, also cover the successful insertion in the standard of care, the chronic administration beyond progression disease, the personalization based on predictors of response and the potential combination with other targeted therapies.

**Characterization of recombinant antibodies for cancer therapy by infrared spectroscopy.** Valdivia AA, Barth A, Batista YR, Kumar S. *Biologicals.* 2013 Mar;41(2):104–10.

Fourier transform infrared (FTIR) spectroscopy was used to study the structure of the recombinant antibodies 1E10, anti-CD20 and hR3, which are used as anti-cancer therapeutic drugs. We tested their sensitivity against different conditions and treatments such as pH, temperature, freeze-thaw cycles and drying, which are relevant for the practical usefulness of the drugs. All antibodies were stable against moderate temperature increases (up to 50 °C) and pH changes (range 5-9). 1E10 was sensitive to extreme pH values (pH 3 and 12), whereas hR3 was most sensitive to temperature (at and above 60 °C). We did not observe any significant changes upon freeze-thaw and drying treatments. The secondary structure content of all three antibodies was estimated to be similar to that of IgG with ~64%  $\beta$ -sheet, 0%  $\alpha$ -helix and ~36% other structure.

**CIGB-247: a VEGF-based therapeutic vaccine that reduces experimental and spontaneous lung metastasis of C57Bl/6 and BALB/c mouse tumors.** Bequet-Romero M, Morera Y, Ayala-Ávila M, Ancizar J, Soria Y, Blanco A, et al. *Vaccine.* 2012 Feb 27;30(10):1790–9.

CIGB-247 is a novel cancer therapeutic vaccine that uses a mutated form of human VEGF as antigen. Being metastatic disease the most dramatic factor of tumor biology affecting patient survival and cure, preclinical evaluation of the impact of CIGB-247 vaccination on experimental metastasis mouse models is highly relevant, and constitutes the focus of this work. CIGB-247 was administered in a weekly schedule known to effectively reduce primary tumor growth. The vaccine was tested in experimental and spontaneous metastasis models of colon (CT26), lung (3LL-D122) and breast (F3II) carcinomas growing in C57Bl/6 or BALB/c mice. Primary tumor growth parameters, metastatic counts, and/or animal survival were recorded. Histology and specific humoral and cellular responses to the vaccine were evaluated. As compared to control groups, CIGB-247 vaccination significantly reduced the number and size of metastatic tumor foci in lungs after intravenous inoculation of CT26 and 3LL-D122 tumor cells. Spontaneous lung dissemination from 3LL-D122 and F3II breast tumor cells implanted in the footpad, or subcutaneously, was also reduced by immunization with CIGB-247. The vaccine elicited in both mouse strains antibodies specific for human and murine VEGF that effectively blocked the interaction of VEGF with VEGF receptor 2. Differing from other experimental reports that describe the use of VEGF for active tumor immunotherapy, CIGB-247 elicited a specific cellular response, measured

both by a DTH increment and the induction of spleen cells cytotoxic to syngeneic tumor cells producing murine VEGF. In summary our results reinforce the potential of CIGB-247 vaccination to reduce both tumor growth and the number and size of tumor metastasis in lungs, the latter both after direct inoculations of cells in the blood stream, or as part of primary tumor progression in immunocompetent mice.

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

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

**[Comparative study of the biodistribution of (99m)Tc-HYNIC-Lys3-Bombesin obtained with the EDDA/tricine and NA/tricine as coligands]**. Hernández-Cairo A, Perera-Pintado A, Prats-Capote A, Batista-Cuellar JF, Casacó-Santana C. Rev Esp Med Nucl Imagen Mol. 2012 Mar–Apr;31(2):61–5. Spanish.

The aim of present investigation was to evaluate biodistribution in healthy animals and in tumor models of the radiopharmaceuticals (99m)Tc-EDDA/tricine-HYNIC-Lys3-Bombesin (HYNIC-Lys3-BN) and (99m)Tc-NA/tricine-HYNIC-Lys3-BN. Biodistribution and pharmacokinetics were carried out over 24 hours. To do so, 24 healthy Wistar rats were used and were administered  $37.0 \pm 0.8$  MBq/rat of each radiopharmaceutical. For the tumor model study, 20 CD-1 nude mice were used and prostate tumors (PC3) were implanted in all the mice. Ten days later, tumor volumes were calculated and  $40.00 \pm 0.04$  MBq/mice of each radiopharmaceutical were injected. Both showed high radiochemical purity:  $98.08 \pm 0.25\%$  for EDDA/tricine product and  $95.1 \pm 0.3\%$  for the conjugate with NA/tricine. Uptake of the radiopharmaceutical with NA/tricine was significantly higher in organs of the reticulo-endothelial system of healthy Wistar rats during 24h, specifically in the liver and spleen. Both labeled compounds showed no significant differences between their blood elimination half lives. Average of tumor growth was  $0.93 \pm 0.02$  cm<sup>3</sup> and affinity for tumors showed a growing and specific binding of both radiopharmaceuticals, although it was significantly higher for the EDDA/tricine conjugate. This outcome made it possible to corroborate the direct relationship between the density of gastrin releasing peptide and its receptors (GRPr) and the variation of the accumulation of the radiopharmaceuticals in the tumor. Use of EDDA/tricine as coligand is more appropriate than NA/tricine for labeling of HYNIC-Lys3-BN with (99m)Tc.

**Computational tools in the discovery of new G-quadruplex ligands with potential anticancer activity.** Castillo-González D, Pérez-Machado G, Pallardó F, Garrigues-Pelufo TM, Cabrera-Pérez MA. Curr Top Med Chem. 2012;12(24):2843–56.

Guanine-rich sequences found at telomeres and oncogenes have the capacity to form G-quadruplex (G4) structures. It has been found a relationship between the ability to stabilizing G4 structures and anticancer activity. Guanine quadruplexes stabilization and its implication in cancer phenomena is a therapeutic target relatively recent. Computer-aided drug design has been a very useful tool for the search of new candidates. In last years, methodologies have improved with the development of the computational sciences. The hardware is also enhanced, new techniques are explored. NMR and X-ray information about different targets are discovered continually. The continuous augmentation of new powerful and comprehensive software's with this purpose is other significant factor that contributes to the discovering of new compounds. Nevertheless computer-aided drug design has not been vastly employed in the design of new compound with G4

stabilization activity. All things considered, this review will be focused on the influence of computational techniques on speeding up the discovery of new G4 ligands.

**Concomitant combination of active immunotherapy and carboplatin- or paclitaxel-based chemotherapy improves anti-tumor response.** Diaz Y, Tundidor Y, Lopez A, Leon K. *Cancer Immunol Immunother.* 2013 Mar;62(3):455–69.

Recent preclinical evidence substantially supports the successful combination of chemotherapies and active immunotherapy for cancer treatment. These data sustain the effect of sequential combination schemes (vaccine plus chemotherapy or vice versa), which could be difficult to implement in clinical practice. Since chemotherapy is the standard treatment for most cancers, ethical issues forbid its delay and make difficult the evaluation of other treatments such as using an immunotherapeutic agent. Besides, vaccines must be applied as soon as possible to advanced cancer patients, in order to give them time to develop an effective immune response. Thus, a clinically attractive scenario is the concomitant application of treatments. However, little is known about the specific effect of different chemotherapeutic agents when combined with a cancer vaccine in such concomitant treatment. In this work, we analyze the influence of high-dose carboplatin or paclitaxel in the generation of a specific immune response when administered concomitantly with an OVA vaccine. Interestingly, neither carboplatin nor paclitaxel affects the humoral and CTL in vivo response generated by the vaccine. Moreover, an enhancement of the overall anti-tumor effect was observed in animals treated with OVA/CF vaccine combined with cytotoxic drugs. Moreover, the effect of the concomitant treatment was tested using a tumor-related antigen, the epidermal growth factor (EGF). Animals administered with EGF-P64k/Montanide and cytotoxic agents showed an antibody response similar to that from control animals. Therefore, our study suggests that carboplatin and paclitaxel can be concomitantly combined with active immunotherapies in the clinical practice of advanced cancer patients.

**Conjunctival metastasis from Kaposi's sarcoma: a case report.** Albin Cano RG, Perez Cardenas JC. *Diagn Cytopathol.* 2011 Feb;39(2):128–31.

We present one case of Kaposi's sarcoma with conjunctive metastasis. The case describes a 71-years-old white male, who was admitted at hospital by malaise, slight pain on the left eyelid, nodular lesion of the left leg, and enlarge lymph nodes in inguinal regions. The conjunctive neoplasms are rare, and we most frequently can see the pigment ones followed by squamous epithelials and lymphomas. The primary Kaposi's sarcoma (KS) from conjunctiva is a rare tumor and it has increased by AIDS emergence and immunosuppression. The appearance of conjunctival metastasis from KS is a stand out event.

**Control of advanced cancer: the road to chronicity.** Lage A, Crombet T. *Int J Environ Res Public Health.* 2011 Mar;8(3):683–97.

Despite the recent trend toward a slight decrease in age-adjusted cancer mortality in some countries, crude mortality rates will continue to increase, driven by the demographic shift towards an aged population. Small molecules (small molecules and biologics) are not only a new therapeutic acquisition, but the tools of a more fundamental transition: the transformation of cancer from a rapidly fatal disease into a chronic condition. Antibodies and cancer vaccines can be used for a long time, even beyond progressive disease, and in aged patients, usually unfit for more aggressive conventional treatments. However, this transition to chronicity will require novel developmental guidelines adequate to this kind of drugs, for which optimal dose is not usually the maximal tolerated dose, pharmacokinetics does not define treatment schedule, and tumor shrinkage is not a good correlate of survival. The ongoing cancer immunotherapy program (including several monoclonal antibodies and therapeutic vaccines) at the Centre of Molecular Immunology can illustrate the issues to be addressed, both biological and social, along the path to transform advanced cancer into a chronic non-communicable disease compatible with years of quality life.

**Cytofluorimetric evaluation of N-glycolylated GM3 ganglioside expression on murine leukocytes.** Miranda A, de León J, Roque-Navarro L, Fernández LE. *Immunol Lett.* 2011 Jun 30;137(1–2):38–45.

Gangliosides are considered relevant components of lipid rafts at the plasma membrane. Antigen encounter, immunological synapse assembly and signal transduction modify lipid raft composition and distribution on immune system cells. On the contrary of other gangliosides, differential expression of the N-glycolylated variant of GM3 (NGcGM3) on murine leukocytes has received limited attention. In particular, whether cell activation modulates the expression of NGcGM3 on lymphoid and myeloid cells is still unexplored. Availability



of the NGcGM3 specific 14F7 MAb allows us to characterize by cytofluorimetric assays the presence of this molecule on resting and activated immune system cells. On T cells, preferential expression of NGcGM3 was detected on CD4(+) single positive thymocytes, peripheral CD4(+) lymphocytes and natural occurring regulatory T cells. In comparison with peritoneal B1 cells, reduced expression of NGcGM3 was observed in peritoneal B2 and splenic B cell subpopulations. Of note, activation of CD4(+) and NK 1.1(+) cells abrogated NGcGM3 expression while LPS-maturated DC increased the ganglioside level at the plasma membrane. Modifications on the presence of NGcGM3 mediated by cell activation did not influence the expression of the N-acetylated variant of GM3 (NAcGM3). In addition to extend previous descriptions of NGcGM3 expression on immunity cell subpopulations, this work highlights the opposite effect of cellular activation over NGcGM3 levels on lymphoid and myeloid cellular series. Obtained results complement the evaluation of a tumor-specific, non-human sialic acid containing ganglioside that has been considered an attractive target for cancer immunotherapy.

**Delineating the functional map of the interaction between nimotuzumab and the epidermal growth factor receptor.** Tundidor Y, García-Hernández CP, Pupo A, Cabrera Infante Y, Rojas G. MAbs. 2014 Jul-Aug;6(4):1013–25.

Molecular details of epidermal growth factor receptor (EGFR) targeting by nimotuzumab, a therapeutic anti-cancer antibody, have been largely unknown. The current study delineated a functional map of their interface, based on phage display and extensive mutagenesis of both the target antigen and the Fv antibody fragment. Five residues in EGFR domain III (R353, S356, F357, T358, and H359T) and the third hypervariable region of nimotuzumab heavy chain were shown to be major functional contributors to the interaction. Fine specificity differences between nimotuzumab and other anti-EGFR antibodies were revealed. Mapping information guided the generation of a plausible *in silico* binding model. Knowledge about the epitope/paratope interface opens new avenues for the study of tumor sensitivity/resistance to nimotuzumab and for further engineering of its binding site. The developed mapping platform, also validated with the well-known cetuximab epitope, allows a comprehensive exploration of antigenic regions and could be expanded to map other anti-EGFR antibodies.

**Effectiveness of adaptive designs for phase II cancer trials.** López MF, Dupuy JF, Gonzalez CV. Contemp Clin Trials. 2012 Jan;33(1):223–7.

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

**Effects of an epidermal growth factor receptor-based cancer vaccine on wound healing and inflammation processes in murine experimental models.** Fuentes D, Chacón L, Casacó A, Ledón N, Fernández N, Iglesias A, et al. Int Wound J. 2014 Feb;11(1):98–103.

Anti-epidermal growth factor receptor (EGFR) therapies have been proven clinically effective for a variety of epithelial tumours. Vaccination of mice with the extracellular domain (ECD) of autologous EGFR overcomes the tolerance to self-EGFR and has antimetastatic effect on EGFR+ tumour. Because EGF/EGFR-signalling plays an important role in the inflammation stage of wound healing, the main objective of this study was to explore the possible role of murine (m) EGFR-ECD vaccine in the croton-oil-induced ear oedema and wound healing process in mice as autologous experimental models, mimicking the possible post-surgical wound complication in patients treated with human EGFR-ECD/VSSP vaccine. Mice were intramuscularly immunised four times; biweekly with the mEGFR-ECD/VSSP/Mont. Seven days later, an 8 mm diameter, full-thickness skin wound was created on the back of each animal. Immunisation induced a strong specific humoral response

against the mEGFR-ECD protein and a DTH dose-response curve but interestingly, animals treated with mEGFR-ECD/VSSP/Mont had similar inflammatory and healing speed responses compared to control ones. These data suggest that application of mEGFR-ECD/VSSP vaccine as a therapeutic approach in cancer patients could not elicit a poor healing process after surgery.

**Effect of blockade of the EGF system on wound healing in patients vaccinated with CIMAvax® EGF.** Fernández Lorente A, Acosta Brooks S, Neninger Vinageras E, Barroso Alvarez Mdel C, Wilkinson Brito B, Troche Concepción M, et al. *World J Surg Oncol*. 2013 Oct 15;11:275.

**Background** The epidermal growth factor receptor (EGFR) signaling system is frequently unbalanced in human malignancies due to increased ligand production, receptor overexpression, receptor mutations, and/or cross-talk with other receptor systems. For this reason, the EGFR is an attractive target for anticancer therapy. The epidermal growth factor also plays an important role in regulating multiple facets of cutaneous wound healing, including inflammation, wound contraction, proliferation, migration, and angiogenesis. In the Center of Molecular Immunology, a cancer vaccine is produced (CIMAvax® EGF) that blocks the binding of EGF to its receptor. This blockade causes a significant inverse association between the anti-EGF antibody titers and EGF concentration. Around 1,500 patients with non-small cell lung cancer have been treated, showing that this vaccine is safe, immunogenic, increases survival and improves quality of life. Taking into account the therapeutic benefits of CIMAvax® EGF vaccination and the role of EGF-EGFR system in the wound healing process, we decided to conduct a retrospective research with the aim of determining the effect to the CIMAvax® EGF vaccine on the wound healing process in patients undergoing surgical treatment. **Methods** Medical records of 452 vaccinated patients were reviewed and only six patients receiving surgical treatment were identified. Further information about these six patients was obtained from source documents, including medical records and operative reports using an observational list that included different variables. Post-surgical wound healing complications were identified using the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTC) version 3.0. **Results** None of the six patients operated on presented adverse events related to the wound healing, that is to say, no wound dehiscence, wound infection, delayed wound healing, fistula formation, abscess formation or hemorrhage/bleeding associated with surgery during treatment with CIMAvax® EGF occurred. **Conclusions** These results suggest that the use of CIMAvax® EGF does not produce a deleterious effect in the wound healing process.

**EGFR-Targeting as a Biological Therapy: Understanding Nimotuzumab's Clinical Effects.** Perez R, Moreno E, Garrido G, Crombet T. *Cancers (Basel)*. 2011 Apr 18;3(2):2014–31.

Current clinical trials of epidermal growth factor receptor (EGFR)-targeted therapies are mostly guided by a classical approach coming from the cytotoxic paradigm. The predominant view is that the efficacy of EGFR antagonists correlates with skin rash toxicity and induction of objective clinical response. Clinical benefit from EGFR-targeted therapies is well documented; however, chronic use in advanced cancer patients has been limited due to cumulative and chemotherapy-enhanced toxicity. Here we analyze different pieces of data from mechanistic and clinical studies with the anti-EGFR monoclonal antibody Nimotuzumab, which provides several clues to understand how this antibody may induce a biological control of tumor growth while keeping a low toxicity profile. Based on these results and the current state of the art on EGFR-targeted therapies, we discuss the need to evaluate new therapeutic approaches using anti-EGFR agents, which would have the potential of transforming advanced cancer into a long-term controlled chronic disease.

**Endoscopic approach to ventricular atrium for biopsy of pineal region tumour: case report.** Hernández-León O, Rodríguez-Villalonga OL, Pérez-Nogueira FR, Guillén-Cánovas EJ, Alvarez-Toledo N, Lemus-Saraceni A. *Neurocirugía (Astur)*. 2014 Jan–Feb;25(1):43–7. Spanish.

**Introduction** The usual endoscopic approach in the management of pineal region tumours consists of inserting the scope into the frontal horn of the lateral ventricle and advancing it through the foramen of Monro into the third ventricle. We report the case of a patient with a pineal tumour on whom we used an endoscopic approach through the ventricular atrium to obtain a biopsy by opening the choroidal fissure. **Clinical case** This young 25-year-old man presented with headache and double vision. Papilloedema and Parinaud's syndrome were found on physical examination. Cranial magnetic resonance revealed a pineal mass and hydrocephalus. We initially performed a third ventriculostomy and a tumour biopsy through a frontal burr hole. The tissue sample was not useful for pathological diagnosis and we decided to perform a second endoscopic biopsy. **Conclusions** The endoscopic approach to pineal region masses, reaching the ventricular atrium

through a parietal burr hole and opening the choroidal fissure, makes it possible to take a biopsy using a single endoscopic approach without needing to cross other ventricular structures.

#### **Endoscopic findings and associated risk factors in primary health care settings in Havana,**

**Cuba.**Galbán E, Arús E, Periles U. MEDICC Rev. 2012 Jan;14(1):30–7.

**Introduction** Upper gastrointestinal endoscopy, traditionally performed in Cuba in specialized hospitals, was decentralized to the primary health care level in 2004 to make it more patient-accessible. **Objectives** Describe frequency and distribution of the principal symptomatic diseases of the upper gastrointestinal tract and their relation to the main risk factors associated with each in a sample of urban adults who underwent upper gastrointestinal endoscopy in primary care facilities in Havana in selected months of 2007. **Methods** A multicenter cross-sectional study was conducted, including 3556 patients seen in the primary health care network of Havana from May through November 2007. The endoscopies were performed at the 22 polyclinics (community health centers) providing this service. Diagnostic quality and accuracy were assessed by experienced gastroenterologists using a validated tool. Patients responded to a questionnaire with clinical, epidemiologic, and sociodemographic variables. Univariate and multivariate analyses (unconditional logistical regression) were used to identify associated risk factors. The significance level was set at  $p < 0.05$  (or confidence interval excluding 1.0). **Results** The diagnoses were: gastritis (91.6%), duodenitis (57.8%), hiatal hernia (46.5%), esophagitis (25.2%), duodenal ulcer (15.8%), gastric ulcer (6.2%) and malignant-appearing lesions (0.4%). Overall prevalence of *Helicobacter pylori* infection was 58.4%. The main risk factors for duodenal ulcer were *H. pylori* infection (OR 2.70, CI 2.17-3.36) and smoking (OR 2.08, CI 1.68-2.58); and for gastric ulcer, *H. pylori* (OR 1.58, CI 1.17-2.15) and age  $\geq 60$  years (OR 1.78, CI 1.28-2.47). *H. pylori* infection was the main risk factor for gastritis (OR 2.29, CI 1.79-2.95) and duodenitis (OR 1.58, CI 1.38-1.82); and age  $\geq 40$  years for hiatal hernia (OR 1.57, CI 1.33-1.84). External evaluation was "very good" or "good" for 99.3% of endoscopic procedures and 97.9% of reports issued. **Conclusions** Gastrointestinal endoscopy performed in primary care yielded high quality results and important information about prevalence of the most common diseases of the upper GI tract and associated risk factors. This study provides a reference for new research and can inform objective recommendations for community-based interventions to prevent and control these diseases. The existence of a network of universally accessible diagnostic endoscopy services at the primary care level, will contribute to conducting further research.

**Epidermal growth factor vaccine in non-small-cell lung cancer.** Gonzalez Marinello GM, Santos ES, Raez LE. Expert Rev Anticancer Ther. 2012 Apr;12(4):439–45.

After many years of uncertainty regarding the role of immunotherapy in cancer, we finally have vaccines approved for the treatment of some malignancies (e.g., prostate cancer and melanoma). In non-small-cell lung cancer, several vaccines are being studied in randomized Phase III clinical trials due to their promising results seen in the clinic, such as BLP-25 and melanoma-associated antigen A3. Traditionally, non-small-cell lung cancer has not been considered a good target for immunotherapy due to lack of immunogenicity and the strong presence of regulatory T cells, which do not allow an adequate immune response in the host. EGF vaccination is a novel area of immunotherapy for this disease. Thus far, there has been success in generating immune and clinical responses with this vaccine in several clinical trials, and we will review in depth the efficacy and toxicity of this novel agent.

#### **[External evaluation of population-based cancer registries: the REDEPICAN Guide for Latin**

**America].** Navarro C, Molina JA, Barrios E, Izarzugaza I, Loria D, Cueva P, et al. Rev Panam Salud Publica. 2013 Nov;34(5):336–42. Spanish.

**Objective** Evaluate the feasibility of the REDEPICAN Guide (Red Iberoamericana de Epidemiología y Sistemas de Información en Cáncer) and its adaptation to the current situation of population-based cancer registries (PBCRs) in Latin America and the Caribbean as a useful tool to improve these registries. **Methods** Experts in cancer registries and health audits designed the guide and developed seven domains to evaluate in PBCRs. Several criteria were selected for each domain, with corresponding standards, scored according to three levels of compliance. Two training courses for external evaluators and three discussion panels for experts were organized. The guide was tested in six PBCRs in Latin America and Spain. **Results** The guide contains 68 criteria, 10 of which are considered essential for a PBCR. Based on its score, a registry is regarded as acceptable (41-199), good (200-299), or excellent (300-350). The registry methods domain accounts for 25% of the score, followed by completeness and validity (19%), dissemination of outcomes (19%), structure (13%), confidentiality and ethical aspects (11%), comparability (9%), and the procedures manual (3%). The pilot project enabled (1) enhancement of criteria and standards, (2) expansion of

the quality concept to include client needs, and (3) strengthening the dissemination of outcomes section. Two of the Latin American registries that were evaluated improved their quality, meeting the standards of the International Agency for Research on Cancer. **Conclusions** Development of the REDEPICAN Guide has taken into account the context of the registries in Latin America and is a useful and innovative tool for improving the quality of PBCRs. Furthermore, it is ready for use in other countries and registries.

**Gallic acid indanone and mangiferin xanthone are strong determinants of immunosuppressive anti-tumour effects of *Mangifera indica* L. bark in MDA-MB231 breast cancer cells.** García-Rivera D, Delgado R, Bougarne N, Haegeman G, Berghe WV. *Cancer Lett.* 2011 Jun 1;305(1):21–31.

Vimang is a standardized extract derived from Mango bark (*Mangifera Indica* L.), commonly used as anti-inflammatory phytomedicine, which has recently been used to complement cancer therapies in cancer patients. We have further investigated potential anti-tumour effects of glucosylxanthone mangiferin and indanone gallic acid, which are both present in Vimang extract. We observed significant anti-tumour effects of both Vimang constituents in the highly aggressive and metastatic breast cancer cell type MDA-MB231. At the molecular level, mangiferin and gallic acid both inhibit classical NFκB activation by IKKα/β kinases, which results in impaired IκB degradation, NFκB translocation and NFκB/DNA binding. In contrast to the xanthone mangiferin, gallic acid further inhibits additional NFκB pathways involved in cancer cell survival and therapy resistance, such as MEK1, JNK1/2, MSK1, and p90RSK. This results in combinatorial inhibition of NFκB activity by gallic acid, which results in potent inhibition of NFκB target genes involved in inflammation, metastasis, anti-apoptosis and angiogenesis, such as IL-6, IL-8, COX2, CXCR4, XIAP, bcl2, VEGF. The cumulative NFκB inhibition by gallic acid, but not mangiferin, is also reflected at the level of cell survival, which reveals significant tumour cytotoxic effects in MDA-MB231 cells. Altogether, we identify gallic acid, besides mangiferin, as an essential anti-cancer component in Vimang extract, which demonstrates multifocal inhibition of NFκB activity in the cancer-inflammation network.

**Gold nanoparticle conjugates: recent advances toward clinical applications.** Cao-Milán R, Liz-Marzán LM. *Expert Opin Drug Deliv.* 2014 May;11(5):741–52.

**Introduction** Gold nanoparticles display a unique combination of chemical inertness, surface chemistry and size- and shape-dependent electronic and optical properties, which render them ideal for clinical applications. **Areas Covered** The present article describes recent advancements on the application of gold nanoparticles in vaccine development and gene therapy, with augmented efficiencies in cell uptake, specific binding to bioreceptors in cells, protection of conjugated biomolecules and so forth. Additionally, we discuss how the electronic structure of the nanoparticles can be exploited for enhanced radiotherapy and X-ray tomography, while their optical properties can be used for photothermal cancer therapy or light-triggered drug delivery systems for enhanced chemotherapy. **Expert Opinion** We analyze certain critical aspects and possible challenges that should be solved in order to use gold nanoparticle conjugates in vaccine research, as well as on the potential combination of properties to improve gene therapy and cancer treatment.

**Human antibodies reactive to NeuGcGM3 ganglioside have cytotoxic antitumor properties.** Rodríguez-Zhurbenko N, Martínez D, Blanco R, Rondón T, Griñán T, Hernández AM. *Eur J Immunol.* 2013 Mar;43(3):826–37.

N-glycosylated gangliosides are not naturally expressed in healthy human tissues but are overexpressed in several tumors. We demonstrate the existence of antibodies that bind (N-glycosylneuraminyl)-lactosylceramide (NeuGcGM3) and are detectable in the sera of 65 from the 100 donors (65%) tested by ELISA. From those 65 NeuGcGM3 antibody-positive donors, 35 had antibodies that were able to recognize and kill NeuGcGM3-expressing tumor cells by a complement-mediated mechanism. After complement inactivation, 11 of the 35 positive sera showed a direct cytotoxic effect on the tumor cells. This complement-independent cytotoxicity was dependent on the presence of antigen on the membrane and resembles an oncotic necrosis cell death. Both the levels of anti-NeuGcGM3 antibodies in the sera as well as the percentage of healthy donors with this immunity decreased with the age of the donor. In contrast to age and gender-matched healthy donors, we could only detect low reactivity against NeuGcGM3 in the sera of six out of 53 non-small cell lung cancer patients. These results suggest the existence of antibodies against NeuGcGM3 with antitumor immune surveillance functions, reinforcing the importance of N-glycosylated gangliosides as antitumor targets.

**Human IL-2 mutein with higher antitumor efficacy than wild type IL-2.** Carmona T, Pacios A, Enamorado M, Moreno E, García-Martínez K, Fuente D, et al. *J Immunol.* 2013 Jun 15;190(12):6230–8.

IL-2 has been used for the treatment of melanoma and renal cell carcinoma, but this therapy has limited efficacy and severe toxicity. Currently, it is assumed that part of the limited efficacy is due to the IL-2-driven preferential expansion of regulatory T cells, which dampen the antitumor immunity. In this study, we characterize a human IL-2 mutant with higher antitumor efficacy and lower toxicity than wild type human IL-2 (wtIL-2). The mutant differs from wtIL-2 by four mutations at the interface with the  $\alpha$  subunit of IL-2R. The IL-2 mutant induces in vitro proliferation of CD8(+)CD44(hi) and NK1.1 cells as efficiently as does wtIL-2, but it shows a reduced capacity to induce proliferation of CD4(+)Foxp3(+) regulatory T cells. The IL-2 mutant shows a higher antimetastatic effect than does wtIL-2 in several transplantable tumor models: the experimental metastasis model of MB16F0 melanoma and the experimental and spontaneous metastasis models for the mouse pulmonary carcinoma 3LL-D1222. Relevantly, the IL-2 mutant also exhibits lower lung and liver toxicity than does wtIL-2 when used at high doses in mice. In silico simulations, using a calibrated mathematical model, predict that the properties of IL-2 mutein are a consequence of the reduction, of at least two orders of magnitude, in its affinity for the  $\alpha$  subunit of IL-2R (CD25). The human IL-2 mutant described in the present work could be a good candidate for improving cancer therapy based on IL-2.

**[Idiotypes as immunogens: facing the challenge of inducing strong therapeutic immune responses against the variable region of immunoglobulins.](#)** López-Requena A, Burrone OR, Cesco-Gaspere M. *Front Oncol.* 2012 Nov 9;2:159. doi: 10.3389/fonc.2012.00159. eCollection 2012.

Idiotype (Id)-based immunotherapy has been exploited as cancer treatment option. Conceived as therapy for malignancies bearing idiotypic antigens, it has been also extended to solid tumors because of the capacity of anti-idiotypic antibodies to mimic Id-unrelated antigens. In both these two settings, efforts are being made to overcome the poor immune responsiveness often experienced when using self immunoglobulins as immunogens. Despite bearing a unique gene combination, and thus particular epitopes, it is normally difficult to stimulate the immune response against antibody variable regions. Different strategies are currently used to strengthen Id immunogenicity, such as concomitant use of immune-stimulating molecules, design of Id-containing immunogenic recombinant proteins, specific targeting of relevant immune cells, and genetic immunization. This review focuses on the role of anti-Id vaccination in cancer management and on the current developments used to foster anti-idiotypic B and T cell responses.

**[Immunohistochemical Reactivity of the 14F7 Monoclonal Antibody Raised against N-Glycolyl GM3 Ganglioside in Some Benign and Malignant Skin Neoplasms.](#)** Blanco R, Rengifo E, Rengifo CE, Cedeño M, Frómata M, Carr A. *ISRN Dermatol.* 2011;2011:848909. doi: 10.5402/2011/848909. Epub 2011 Apr 10.

The evaluation of 14F7 Mab (anti-N-glycolyl GM3 ganglioside) immunorecognition in normal skin, cutaneous malignant melanoma (CMM), and in lymph node metastases (LNM) has been previously reported. In this work we extended the study to benign (BMN) and dysplastic (DMN) melanocytic nevi, basal (BCC), and squamous cell carcinoma (SCC). Immunohistochemical assays with 14F7 followed by a biotinylated link universal and streptavidin-AP in normal and pathological tissues were made. No reaction of 14F7 in normal skin (0/10) as well as a low reactivity in BMN (2/11) and DMN (1/7) was detected. A limited staining in BCC (2/13) and in SCC (4/8) was also evidenced, while 14F7 Mab were mostly reactive in CMM (28/28) and in LNM (6/7). These results suggest that 14F7 reactivity could be closely related with the more aggressive biological behavior of CMM and also support the use of NeuGcGM3 as target for both passive and active melanoma immunotherapy.

**[Immunopharmacology 2011: an updated report of clinical achievements and perspectives.](#)** Landys Chovel M, Perea S, de los Ángeles Robinson M, Rodeiro I, Delgado R, Ochoa RF, et al. *Expert Rev Clin Pharmacol.* 2011 Nov;4(6):693–5.

On 26-30 June 2011 the Cuban Society of Pharmacology organized the Second International Congress on Immunopharmacology (Immunopharmacology 2011), held at the beautiful Convention Centre 'Plaza América' and the Meliá Varadero Hotel, in Varadero beach, Cuba. The main topics of the congress were immunopharmacology (including inflammation, cancer immunotherapy and immunomodulation), neuroimmunology, and the pharmacology of cytochrome P450 and transporters, among other relevant and updated related topics. Immunopharmacology 2011 offered an outstanding scientific program with the active contribution of 90 speakers from 23 foreign countries, as well as more than 170 Cuban researchers from the most important local institutions devoted to the development of immunology and pharmacology sciences.

**Immunoreactivity of the 14F7 Mab (Raised against N-Glycolyl GM3 Ganglioside) as a Positive Prognostic Factor in Non-Small-Cell Lung Cancer.** Blanco R, Rengifo CE, Cedeño M, Frómeta M, Rengifo E, Carr A. *Patholog Res Int.* 2012;2012:235418. doi: 10.1155/2012/235418. Epub 2012 Feb 26.

Lung carcinoma is the leading cause of cancer-related mortality worldwide. Therefore, numerous studies are focusing on the assessment of other biological and molecular prognostic factors in these tumors. We evaluated the relationship between 14F7 Mab reactivity, pathological features, DNA-content and S-phase fraction (SPF), and their impact in the survival of NSCLC patients. Hematoxylin and eosin staining and immunohistochemistry optical microscopy assays as well as DNA content and SPF measuring using flow cytometry were performed. The 14F7 reactivity was widely observed in NSCLC sections, no depending of the clinicopathological characteristics. We also obtained differences in the intensity of reaction with 14F7 as well as in the SPF between diploid and aneuploid carcinomas. Patients with diploid tumors showing higher SPF and 14F7 reaction joint to a low mitotic index displayed higher survival rates. Our results are in agreement with the assumption of the possible positive prognostic value of 14F7 staining in NSCLC.

**Induction of immunogenic apoptosis by blockade of epidermal growth factor receptor activation with a specific antibody.** Garrido G, Rabasa A, Sánchez B, López MV, Blanco R, López A, et al. *J Immunol.* 2011 Nov 15;187(10):4954–66.

Despite promising results in the use of anti-epidermal growth factor receptor (EGFR) Abs for cancer therapy, several issues remain to be addressed. An increasing emphasis is being placed on immune effector mechanisms. It has become clear for other Abs directed to tumor targets that their effects involve the adaptive immunity, mainly by the contribution of Fc region-mediated mechanisms. Given the relevance of EGFR signaling for tumor biology, we wonder whether the oncogene inhibition could contribute to Ab-induced vaccine effect. In a mouse model in which 7A7 (an anti-murine EGFR Ab) and AG1478 (an EGFR-tyrosine kinase inhibitor) displayed potent antimetastatic activities, depletion experiments revealed that only in the case of the Ab, the effect was dependent on CD4(+) and CD8(+) T cells. Correspondingly, 7A7 administration elicited a remarkable tumor-specific CTL response in hosts. Importantly, experiments using 7A7 F(ab')(2) suggested that in vivo Ab-mediated EGFR blockade may play an important role in the linkage with adaptive immunity. Addressing the possible mechanism involved in this effect, we found quantitative and qualitative differences between 7A7 and AG1478-induced apoptosis. EGFR blocking by 7A7 not only prompted a higher proapoptotic effect on tumor metastases compared with AG1478, but also was able to induce apoptosis with immunogenic potential in an Fc-independent manner. As expected, 7A7 but not AG1478 stimulated exposure of danger signals on tumor cells. Subcutaneous injection of 7A7-treated tumor cells induced an antitumor immune response. This is the first report, to our knowledge, of a tumor-specific CTL response generated by Ab-mediated EGFR inhibition, suggesting an important contribution of immunogenic apoptosis to this effect.

**Induction of leukocyte infiltration at metastatic site mediates the protective effect of NGcGM3-based vaccine.** Labrada M, Pablos I, Prete F, Hevia G, Clavell M, Benvenuti F, et al. *Hum Vaccin Immunother.* 2014 May 27;10(8). [Epub ahead of print]

While the NGcGM3/VSSP vaccine, a preparation consisting in very small sized proteoliposomes (VSSP) obtained by the incorporation of the NGcGM3 ganglioside into the outer membrane protein (OMP) complex of *Neisseria meningitidis*, is currently studied in late stage clinical trials in breast cancer and melanoma patients, mechanisms involved in the vaccine's antitumor effect are insufficiently understood. Here we have addressed the role of adaptive and innate immune cells in mediating the protective effect of the vaccine. To this aim we selected the 3LL-D122 Lewis lung spontaneous metastasis model. Unexpectedly, inoculation of the vaccine in tumor bearing C57BL/6 mice, either by subcutaneous (sc) or intraperitoneal (ip) routes, induced similar anti-metastatic effect. Regardless the T-independent nature of NGcGM3 ganglioside as antigen, the antimetastatic effect of NGcGM3/VSSP is dependent of CD4+ T cells. In a further step we found that the vaccine was able to promote the increase, maturation, and cytokine secretion of conventional DCs and the maturation of Bone Marrow-derived plasmacytoid DCs. In line with this result the in vivo IFN $\alpha$  serum level in ip vaccinated mice increased as soon as 2h after treatment. On the other hand the infiltration of NK1.1+CD3- and NK1.1+CD3+ cells in lungs of vaccinated mice was significantly increased, compared with the presence of these cells in control animal lungs. In the same way NGcGM3/VSSP mobilized acquired immunity effector cells into the lungs of vaccinated tumor bearing mice. Finally and not less noteworthy, leukocyte infiltration in lungs of tumor bearing mice correlates with vaccine induced inhibition of lung metastization.

**In silico studies of potential phosphoresidues in the human nucleophosmin/B23: its kinases and related biological processes.** Ramos-Echazábal G, Chinea G, García-Fernández R, Pons T. J Cell Biochem. 2012 Jul;113(7):2364–74.

Human nucleophosmin/B23 is a phosphoprotein involved in ribosome biogenesis, centrosome duplication, cancer, and apoptosis. Its function, localization, and mobility within cells, are highly regulated by phosphorylation events. Up to 21 phosphosites of B23 have been experimentally verified even though the corresponding kinase is known only for seven of them. In this work, we predict the phosphorylation sites in human B23 using six kinase-specific servers (KinasePhos 2.0, PredPhospho, NetPhosK 1.0, PKC Scan, pkaPS, and MetaPredPS) plus DISPHOS 1.3, which is not kinase specific. The results were integrated with information regarding 3D structure and residue conservation of B23, as well as cellular localizations, cellular processes, signaling pathways and protein-protein interaction networks involving both B23 and each predicted kinase. Thus, all 40 potential phosphosites of B23 were predicted with significant score (>0.50) as substrates of at least one of 38 kinases. Thirteen of these residues are newly proposed showing high susceptibility of phosphorylation considering their solvent accessibility. Our results also suggest that the enzymes CDKs, PKC, CK2, PLK1, and PKA could phosphorylate B23 at higher number of sites than those previously reported. Furthermore, PDK, GSK3, ATM, MAPK, PKB, and CHK1 could mediate multisite phosphorylation of B23, although they have not been verified as kinases for this protein. Finally, we suggest that B23 phosphorylation is related to cellular processes such as apoptosis, cell survival, cell proliferation, and response to DNA damage stimulus, in which these kinases are involved. These predictions could contribute to a better understanding, as well as addressing further experimental studies, of B23 phosphorylation.

**Introduction of imatinib as first-line therapy for chronic myeloid leukemia in Cuba.** Morán VP, Baute RG, Facundo JC, Ramírez PH, Núñez AA, Martínez EE, et al. MEDICC Rev. 2011 Jan;13(1):35–40.

**Introduction** Chronic myeloid leukemia is the first malignant disease to be associated with a genetic lesion and is the first leukemia to provide a genotype model conducive to targeted molecular therapy. It is a chronic clonal myeloproliferative disorder, originating in a pluripotent stem cell common to all three hematopoietic lineages, characterized by overproduction of myeloid cells in all stages of maturation. Approval of the use of imatinib in the United States in 2001 and its introduction in the treatment of chronic myeloid leukemia changed the evolution and prognosis of the disease and began the era of molecular therapy for malignancies. Imatinib is highly effective and causes fewer adverse reactions than earlier treatments based on interferon and hydroxyurea. In Cuba, chronic myeloid leukemia has been treated with interferon since 1998. Starting in 2003, imatinib was gradually introduced for use in newly-diagnosed chronic myeloid leukemia patients. **Objective** Evaluate the use of imatinib as first-line therapy for chronic myeloid leukemia in a group of Cuban patients, based on hematologic, cytogenetic, and molecular response; overall and event-free survival rates; and most frequency and severity of adverse reactions. **Methods** During May 2003 to May 2008, 33 newly-diagnosed chronic myeloid leukemia patients (25 adults, 8 children; <6 months from diagnosis) received a single daily oral dose of imatinib 400 mg from the time of study enrollment. Variables used: (1) to evaluate treatment efficacy: hematologic, cytogenetic, and molecular response; overall and event-free survival; and (2) to evaluate safety: presence of adverse reactions leading to definitive interruption of treatment or death. **Results** Complete hematologic response occurred in 100% of patients, major cytogenetic response in 90.9%, and complete cytogenetic response in 48.5%. Molecular response occurred in 36.4% of patients. With a mean follow-up of 39 months, overall survival was 96% and estimated five-year event-free survival was 85%. No adverse reactions occurred in 39.5% of patients. Adverse reactions most frequently observed were myelosuppression (24.2%) and digestive disorders (21.2%). These were followed, in decreasing order, by edema, primarily orbital (9.1%), skin depigmentation (3%), and cardiac arrhythmias (3%). **Conclusions** In the present study, imatinib was effective first-line therapy for patients with newly-diagnosed chronic myeloid leukemia, as determined by overall and event-free survival rates. No severe adverse reactions were observed.

**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.

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

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

**Lentiviral vector followed by protein immunisation breaks tolerance against the self-antigen Her1 and results in lung cancer immunotherapy.** Aguiar Alpizar Y, Karwacz K, Arce F, Yglesias Rivera A, Fernández LE, Collins MK, et al. *J Gene Med*. 2012 Mar;14(3):151–7.

**Background** Lung cancer remains a leading cause of cancer mortality, and so the aim of the present study was to develop a therapeutic vaccine protocol. **Methods** We constructed a lentiviral vector (LV) expressing the extracellular domain (ECD) of murine Her1, an antigen associated with poor prognosis in lung cancer. **Results** A single LV injection, followed by two Her1 protein boosts, was effective in reducing the metastatic burden of Lewis lung carcinoma in mice. The Her1 LV immunisation generated CD8+ T cells that recognised Her1 ECD presented by dendritic cells and that also homed to Her1-expressing tumours. Protein boosting further increased the CD8+ T cell response and generated anti-Her1 antibodies; in the antibody response, Her1 LV priming increased Th1-dependent immunoglobulin G2c production. **Conclusions** The ability of this vaccine protocol to break both T cell and B cell tolerance to a self-antigen likely explains its effectiveness.

**Linking oncogenesis and immune system evasion in acquired resistance to EGFR-targeting antibodies: Lessons from a preclinical model.** Garrido G, Rabasa A, Sánchez B. *Oncoimmunology*. 2013 Dec 1;2(12):e26904.

Searching for biomarkers that associated with the acquired resistance of malignant cells to epidermal growth factor receptor (EGFR)-targeting monoclonal antibodies is crucial to improve the clinical benefits of these therapeutic agents. We have recently demonstrated that molecular alterations in both oncogenic and immunological pathways may be responsible for such an insensitivity. Our findings suggest that a combination of targeted anticancer agents and immunomodulatory drugs may be useful for overcoming the acquired resistance of cancer cells to EGFR-specific monoclonal antibodies.

**Lonely killers: effector cell- and complement-independent non-proapoptotic cytotoxic antibodies inducing membrane lesions.** Fernández-Marrero Y, López-Requena A. *MAbs*. 2011 Nov–Dec;3(6):528–34.

The majority of the most effective monoclonal antibodies (mAbs) currently in the clinics bind to cancer or immune cells. Classic mechanisms of cell killing by therapeutic mAbs include antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity and induction of apoptosis by engagement of specific cell ligands. A few reports have described mAbs whose cytotoxic activity is Fc-independent and that do not induce the morphological and biochemical changes associated with the apoptosis-type of cell death. Even fewer works describe mAbs able to directly induce membrane lesions. Here, we discuss the available data on those molecules and their cell killing activity, with particular attention to the case of a mAb specific for the tumor-associated N-glycolyl (Neu5Gc)-GM3 ganglioside (GM3(Neu5Gc)). Some similarities are found in the



cell death pathways triggered by these mAbs, but data are not abundant. We conclude that the usefulness of mAbs with a direct cytotoxic activity for immunotherapeutic strategies deserves deeper research.

**Long-term survival in cerebellar glioblastoma multiforme. Case report.** Hernandez-Gonzalez G, Marchione P, De Angelis F, Giannone C, Kouleridou A, Spallone A. *J Neurosurg Sci.* 2012 Dec;56(4):379–81.

Primary cerebellar glioblastoma multiforme (GBM) is a rare tumor in adults that accounts for just 1% of all cases of GBM. Cerebellar GBMs are not yet completely understood also as far as the prognosis. We report a case of cerebellar GBM in a 27-year-old woman. Magnetic resonance imaging (MRI) showed a 3x3.6 cm-sized, ill-defined, heterogeneously enhancing mass in the left cerebellum. GBM was histologically confirmed following radical surgery. Postoperative radiotherapy with concomitant and adjuvant temozolomide chemotherapy was subsequently administered. She has no evidence of recurrence and is in good clinical conditions up-to date, three years after surgery.

**Metronomic Cyclophosphamide and Methotrexate Chemotherapy Combined with 1E10 Anti-Idiotypic Vaccine in Metastatic Breast Cancer.** Soriano JL, Batista N, Santiesteban E, Lima M, González J, García R, et al. *Int J Breast Cancer.* 2011;2011:710292. doi: 10.4061/2011/710292. Epub 2011 Jul 14.

The use of low doses of cytotoxic agents continuously for prolonged periods is an alternative for the treatment of patients with metastatic breast cancer who have developed resistance to conventional chemotherapy. The combination of metronomic chemotherapy with therapeutic vaccines might increase the efficacy of the treatment. Twenty one patients with metastatic breast cancer in progression and a Karnofsky index  $\geq 60\%$ , were treated with metronomic chemotherapy (50 mg of cyclophosphamide orally daily and 2.5 mg of methotrexate orally bi-daily), in combination with five bi-weekly subcutaneous injections of 1 mg of aluminum hydroxide-precipitated 1E10 anti-idiotypic MAb (1E10-Alum), followed by reimmunizations every 28 days. Five patients achieved objective response, eight showed stable disease and eight had disease progression. Median time to progression was 9,8 months, while median overall survival time was 12,93 months. The median duration of the response (CR+PR+SD) was 18,43 months (12,20-24,10 months), being higher than 12 months in 76,9% of the patients. Overall toxicity was generally mild. Metronomic chemotherapy combined with 1E10-Alum vaccine immunotherapy might be a useful therapeutic option for the treatment of metastatic breast cancer due to its potential impact on survival and patient quality of life, low toxicity and advantages of the administration.

**Morphogenesis and aggressiveness of cervix carcinoma.** Izquierdo-Kulich E, Amigó de Quesada M, Pérez-Amor CM, Nieto-Villar JM. *Math Biosci Eng.* 2011 Oct 1;8(4):987–97.

The regular nutritional intake of an expectant mother clearly affects the weight development of the fetus. Assuming the growth of the fetus follows a deterministic growth law, like a logistic equation, albeit dependent on the nutritional intake, the ideal solution is usually determined by the birth-weight being pre-assigned, for example, as a percentage of the mother's average weight. This problem can then be specified as an optimal control problem with the daily intake as the control, which appears in a Michaelis-Menten relationship, for which there are well-developed procedures to follow. The best solution is determined by requiring minimum total intake under which the preassigned birth weight is reached. The algorithm has been generalized to the case where the fetal weight depends in a detailed way on the cumulative intake, suitably discounted according to the history. The optimality system is derived and then solved numerically using an iterative method for the specific values of parameter. The procedure is generic and can be adapted to any growth law and any parameterisation obtained by the detailed physiology.

**NGcGM3/VSSP vaccine as treatment for melanoma patients.** Pérez K, Osorio M, Hernández J, Carr A, Fernández LE. *Hum Vaccin Immunother.* 2013 Jun;9(6):1237–40.

Gangliosides are glycosphingolipids that are present in the plasma membranes of vertebrates and are involved in multiple cellular processes. In the Center of Molecular Immunology an NGcGM3 ganglioside based vaccine has been developed and is conceptualized as a targeted therapy in cancer. NGcGM3/VSSP vaccine had been used as treatment of metastatic melanoma patients and had showed to be safe and immunogenic. The treatment improved antitumoral response or maintain the response obtained with previous onco-specific treatment as chemotherapy. The results indicate that the vaccine improved overall survival of metastatic melanoma patients after first line-chemotherapy. The clinical trial ongoing currently will allow corroborating these results.

**Nimotuzumab in combination with radiotherapy in high grade glioma patients: a single institution experience.** Solomon MT, Miranda N, Jorrín E, Chon I, Marinello JJ, Alert J, et al. *Cancer Biol Ther.* 2014 May;15(5):504–9.

Nimotuzumab, a humanized antibody targeting epidermal growth factor receptor, has potent anti-proliferative, anti-angiogenic, and pro-apoptotic effects in vitro and in vivo. It also reduces the number of radio-resistant CD133(+) glioma stem cells. The antibody has been extensively evaluated in patients with advanced head and neck, glioma, lung, esophageal, pancreatic, and gastric cancer. In this single institution experience, 35 patients with anaplastic astrocytoma (AA) or glioblastoma multiforme (GBM) were treated with irradiation and 200 mg doses of nimotuzumab. The first 6 doses were administered weekly, together with radiotherapy, and then treatment continued every 21 days until 1 year. The median number of doses was 12, and the median cumulative dose was thus 2400 mg of nimotuzumab. The most frequent treatment-related toxicities were increase in liver function tests, fever, nausea, anorexia, asthenia, dizziness, and tremors. These adverse reactions were classified as mild and moderate. The median survival time was 12.4 mo or 27.0 mo for patients with GBM or AA patients, respectively, who received curative-intent radiotherapy in combination with the antibody. The survival time of a matched population treated at the same hospital with irradiation alone was decreased (median 8.0 and 12.2 mo for GBM and AA patients, respectively) compared with that of the patients who received nimotuzumab and curative-intent radiotherapy. We have thus confirmed that nimotuzumab is a very well-tolerated drug, lacking cumulative toxicity after maintenance doses. This study, in a poor prognosis population, validates the previous data of survival gain after combining nimotuzumab and radiotherapy, in newly diagnosed high-grade glioma patients.

**Non-clinical immuno-toxicological evaluation of HER1 cancer vaccine in non-human primates: a 12-month study.** Barro AM, Rivero AI, Gofii AL, Navarro BO, Angarica MM, Ramírez BS, et al. *Vaccine.* 2012 Dec 17;31(1):89–95.

Human epidermal growth factor receptor (HER1) constitutes a tumor associated antigen. Its overexpression in many epithelial tumors has been associated with bad prognosis and poor survival. Cancer vaccine based on the extracellular domain (ECD) of HER1 and adjuvated in very small sized proteoliposomes (VSSP) and Montanide ISA 51-VG is a new and complementary approach for the treatment of epithelial tumors. The present study deals with the immunogenicity of this vaccine in *Macaca fascicularis* monkeys and evaluation of its toxicity during 12 months. Twelve monkeys were randomized into two groups of 3 animals per sex: control and vaccinated. Treated monkeys received 9 doses of vaccination and were daily inspected for clinical signs. Body weight, rectal temperature, cardiac and respiratory rates were measured during the study. Humoral immune response, clinical pathology parameters and delayed type hypersensitivity were analyzed. Skin biopsy was performed at the end of the study in all animals. Animal's survival in the study was 100% (n=12). Local reactions were observed at the administration site of four treated animals (n=6), with two showing slight inflammatory cutaneous damage. Clinical pathology parameters were not affected. HER1 vaccine induced high IgG antibodies titers in the treated animals even when DTH was not observed. The induced antibodies recognized HER1+ tumor cell lines, decreased HER1 phosphorylation and showed anti-proliferative and pro-apoptotic effects in H125 cells. In general the present study showed that HER1 vaccine induced specific immune response in *M. fascicularis* monkeys and was well tolerated, suggesting it could be safely used in clinical studies in epithelial cancer patients.

**Oncogenic transformation tunes the cross-talk between mesenchymal stem cells and T lymphocytes.** Sánchez N, Miranda A, Funes JM, Hevia G, Pérez R, de León J. *Cell Immunol.* 2014 May–Jun;289(1–2):174–84.

Stem cells from mesenchymal origin (MSC) exert a plethora of immunomodulatory effects. We created a neoplastic model based on in vitro step-wise transformation to assess whether oncogenic pathways have the capacity to mould the cross-talk of MSC and lymphocytes. Neoplastic MSC exhibit an increased inhibitory effect on T cell proliferation, either directly or mediated by myeloid derived suppressor cells. Additionally, transformation of MSC enhances T cell apoptosis without reducing either the percentage of CD25 expressing cells or the level of this protein expression. Malignant transformation drives MSC to lose dependency on nitric oxide for immunosuppression whilst increasing the constitutive production of PGE2. Our results indicate that oncogenesis tunes the interplay between MSC and immune cells, favoring cancer immune evasion.

**On the cytotoxic activity of Pd(II) complexes of N,N-disubstituted-N'-acyl thioureas.** Plutín AM, Mocelo R, Alvarez A, Ramos R, Castellano EE, Cominetti MR, et al. *J Inorg Biochem.* 2014 May;134:76–82.

The rational design of anticancer drugs is one of the most promising strategies for increasing their cytotoxicity and for minimizing their toxicity. Manipulation of the structure of ligands or of complexes represents a strategy for which is possible to modify the potential mechanism of their action against the cancer cells. Here we present the cytotoxicity of some new palladium complexes and our intention is to show the importance of non-coordinated atoms of the ligands in the cytotoxicity of the complexes. New complexes of palladium (II), with general formulae  $[Pd(PPh_3)_2(L)]PF_6$  or  $[PdCl(PPh_3)(L)]$ , where  $L=N,N$ -disubstituted- $N'$ -acyl thioureas, were synthesized and characterized by elemental analysis, molar conductivity, melting points, IR, NMR( $^1H$ ,  $^{13}C$  and  $^{31}P\{^1H\}$ ) spectroscopy. The spectroscopic data are consistent with the complexes containing an O, S chelated ligand. The structures of complexes with  $N,N$ -dimethyl- $N'$ -benzoylthiourea,  $N,N$ -diphenyl- $N'$ -benzoylthiourea,  $N,N$ -diethyl- $N'$ -furoylthiourea, and  $N,N$ -diphenyl- $N'$ -furoylthiourea were determined by X-ray crystallography, confirming the coordination of the ligands with the metal through sulfur and oxygen atoms, forming distorted square-planar structures. The  $N,N$ -disubstituted- $N'$ -acyl thioureas and their complexes were screened with respect to their antitumor cytotoxicity against DU-145 (human prostate cancer cells), MDA-MB-231 (human breast cancer cells) and their toxicity against the L929 cell line (health cell line from mouse).

**On the safety of persons accompanying nuclear medicine patients.** Díaz Barreto M, López Bejerano GM, Varela Corona C, Fleitas Estévez I. *Radiat Prot Dosimetry*. 2012 Dec;152(4):313–6.

The presence of caretakers/comforters during nuclear medicine examinations is relatively common. These caretakers receive higher doses than the general public, who receive only environmental/background exposure. The aim of this research was to know about the doses received by two significant groups of caretakers: comforters of cancer patients (Group I) and mothers of small children (Group II). The patients were scheduled to undergo two different diagnostic studies: Immuno-Scintigraphy using a monoclonal antibody bound to  $(99m)Tc$  (for adults) and Renal Scintigraphy using  $(99m)Tc$ -dimercaptosuccinic acid (for children). The average effective doses were 0.27 and 0.29 mSv for Groups I and II, respectively. Additionally, environmental monitoring was performed in the waiting room for injected patients (Room I) and inside the procedure room (Room II). Equivalent environmental doses of 0.28 and 0.24 mSv for Rooms 1 and II, respectively, were found, which are similar to values reported by other authors.

**Phase I clinical trial of the  $(^{131}I)$ -labeled anticarcinoembryonic antigen CIGB-M3 multivalent antibody fragment.** González GP, García IG, González JG, Sánchez LP, Mirabal MV, Marín CC, et al. *Cancer Biother Radiopharm*. 2011 Jun;26(3):353–63.

Center for Genetic Engineering and Biotechnology (CIGB)-M3 is a trivalent recombinant single-chain Fv antibody fragment specific for carcinoembryonic antigen (CEA). Preclinical studies with radiolabeled CIGB-M3 have showed that the antibody fragment accumulates in human colon tumor xenografts growing in nude mice. A Phase I clinical trial was carried out to determine safety, biodistribution, and pharmacokinetics of the radiolabeled CIGB-M3 in two groups of patients with CEA+ colorectal cancers. Group I (10 patients) received a single intravenous injection of 0.3 mg of  $(^{131}I)$ -CIGB-M3 (16.7-23.3 mCi/mg). Group II (7 patients) received 1 mg (5-7 mCi/mg). No adverse events related to the injected product were recorded, and no immunology response was detected up to 6 months after the injection. Tumors were detected in 15 of the 17 studied cases. The pharmacokinetic profile showed beta half-times of 14.1 and 6.3 hours for Groups I and II, respectively. Seventy-two (72) hours after the administration of the product, 85% of the total injected activity was excreted in urine in the form of free  $(^{131}I)$ . The kidneys were identified as the organs that can limit the maximum tolerated dose. The  $(^{131}I)$ -CIGB-M3 was safe in patients with colorectal cancer. The biodistribution and pharmacokinetic data suggest that the product can be further tested for molecular radiotherapy of CEA+tumors.

**Phenotypic heterogeneity in the NCI-H125 cell line affects biological activity using the epidermal growth factor receptor as target.** Blanco R, Cedeño M, González N, Rodríguez R, Sánchez J, Rengifo E. *Acta Pharm*. 2012 Dec;62(4):581–91.

We evaluated the influence of some morphological changes of the NCI-H125 cell line in surface expression of the epidermal growth factor receptor (EGFR) and their impact on some biological activity assays using this molecule as target. Hematoxylin and eosin (H/E) staining, light microscopy immunocytochemistry, flow cytometric antibody-receptor binding test, cell viability determination and cell cycle analysis were performed. Phenotypic changes and inconsistency in EGFR expression were detected in NCI-H125 cell cultures. A significant reduction in the growth rate, mainly characterized by cell cycle arrest in the G<sub>0</sub>-G<sub>1</sub> phase, was also evidenced. Differential distribution of cell viability in NCI-H125 subpopulations and its relationship with the EGFR surface expression were determined. Nuclear alterations observed in NCI- H125 were not apoptosis related. A lack of control of cell cultures affects the reliability and reproducibility of biomedical and

biotechnological research using EGFR as target. Therefore, rigorous control of the above mentioned parameters in these experiments is recommended.

**Physicochemical and biological characterization of 1E10 anti-idiotypic vaccine.** Machado YJ, Rabasa Y, Montesinos R, Cremata J, Besada V, Fuentes D, et al. BMC Biotechnol. 2011 Nov 22;11:112.

**Background** 1E10 monoclonal antibody is a murine anti-idiotypic antibody that mimics N-glycolyl-GM3 gangliosides. This antibody has been tested as an anti-idiotypic cancer vaccine, adjuvated in Al(OH)<sub>3</sub>, in several clinical trials for melanoma, breast, and lung cancer. During early clinical development this mAb was obtained in vivo from mice ascites fluid. Currently, the production process of 1E10 is being transferred from the in vivo to a bioreactor-based method. **Results** Here, we present a comprehensive molecular and immunological characterization of 1E10 produced by the two different production processes in order to determine the impact of the manufacturing process in vaccine performance. We observed differences in glycosylation pattern, charge heterogeneity and structural stability between in vivo-produced 1E10 and bioreactor-obtained 1E10. Interestingly, these modifications had no significant impact on the immune responses elicited in two different animal models. **Conclusions** Changes in 1E10 primary structure like glycosylation; asparagine deamidation and oxidation affected 1E10 structural stability but did not affect the immune response elicited in mice and chickens when compared to 1E10 produced in mice.

**Pilot study of a novel combination of two therapeutic vaccines in advanced non-small-cell lung cancer patients.** Herrera ZM, Ramos TC. Cancer Immunol Immunother. 2014 Jul;63(7):737–47.

Cancer vaccines contain tumor antigens in a pro-inflammatory context with the purpose to generate potent antitumor immune responses. However, tumor cells develop different immunosuppressive mechanisms that limit the effectiveness of an anticancer immune response. Therefore, therapeutic vaccine treatment alone is usually not sufficient to generate tumor regression or survival improvement, especially in the advanced disease scenario in which most clinical studies have been conducted. Combining cancer vaccines with different anticancer therapies such as chemotherapy, radiotherapy and other immunotherapeutic agents has had different levels of success. However, the combination of cancer vaccines with different mechanisms of action has not been explored in clinical trials. To address this issue, the current review summarizes the main clinical and immunological results obtained with two different therapeutic vaccines used in advanced non-small-cell lung cancer patients, inducing an immune response against epidermal growth factor (CIMA<sub>vax</sub>-EGF) and NGcGM3 ganglioside (racotumomab). We also discuss preliminary findings obtained in a trial of combination of these two vaccines and future challenges with these therapies.

**[Postraumatic epidural arachnoid spinal cyst: case report]**. Hernández-León O, Pérez-Nogueira FR, Corrales N. Neurocirugia (Astur). 2011 Jun;22(3):267–70. Spanish.

**Introduction** Extradural arachnoid spinal cysts are infrequent lesions that are associated with spinal trauma, surgery and less frequently with congenital anomalies. The clinical manifestations are similar to those seen with other compressive spinal cord lesions. Magnetic resonance techniques allow to diagnose correctly this pathology and to define its topographic situation. The pathologic history of the patient is essential to establish the etiology. Surgery is the elective treatment in most cases. **Clinical case** The patient is a 35 years old man who has a medical history of penetrating spinal trauma two years ago. In that instance he suffered an unilateral spinal cord section at D2-D3 level with the corresponding Brown Sequard syndrome. A small wound was detected at the skin dorsal level and it was closed without difficulties. At the beginning, he improved his motor right leg function with rehabilitation and vitamins. After two years of good recovery he came to our hospital suffering a neurological deterioration of six months of evolution. The physical examination revealed a spastic paraparesis. Magnetic resonance was performed demonstrating a cystic extradural collection compressing the spinal cord at D3-D4 level. Surgical decompressive treatment allowed to excise the cyst and it was possible to define a dural tear that was closed successfully. The outcome was good with restoration of the initial motor function that he had after the spinal trauma. **Conclusions** Surgical management of postraumatic epidural arachnoid spinal cyst allows to detect the meningeal tear and to close it, which is highly effective on these kinds of lesions.

**Prognostic Role of 14F7 Mab Immunoreactivity against N-Glycolyl GM3 Ganglioside in Colon Cancer.** Lahera T, Calvo A, Torres G, Rengifo CE, Quintero S, Arango Mdel C, et al. J Oncol. 2014;2014:482301.

**Purpose** To assess the prognostic role of 14F7 Mab immunoreactivity, against N-Glycolyl GM3 ganglioside, in patients with colon cancer (CC) and to evaluate the relationship between its expression and clinicopathological features. **Methods** Paraffin-embedded specimens were retrospectively collected from 50 patients with CC operated between 2004 and 2008. 14F7 Mab staining was determined by immunohistochemistry technique and its relation with survival and clinicopathologic features was evaluated. **Results** The reactivity of 14F7 Mab was detected in all cases. Most cases had high level of immunostaining (70%) that showed statistical correlation with TNM stage ( $P = 0.025$ ). In univariate survival analysis, level of 14F7 Mab immunoreactivity ( $P = 0.0078$ ), TNM Stage ( $P = 0.0007$ ) and lymphovascular invasion ( $P = 0.027$ ) were significant prognostic factors for overall survival. Among these variables, level of 14F7 Mab immunoreactivity (HR = 0.268; 95% CI 0.078-0.920;  $P = 0.036$ ) and TNM stage (HR = 0.249; 95% CI 0.066-0.932;  $P = 0.039$ ) were independent prognostic factors on multivariate analysis. **Conclusions** This study is the first approach on the prognostic significance of 14F7 Mab immunoreactivity in patients with colon adenocarcinoma and this assessment might be used in the prognostic estimate of CC, although further studies will be required to validate these findings.

**Prolonged use of nimotuzumab in children with central nervous system tumors: safety and feasibility.** Cabanas R, Saurez G, Alert J, Reyes A, Valdes J, Gonzalez MC, et al. Cancer Biother Radiopharm. 2014 May;29(4):173–8.

Primary brain tumors constitute the most frequent solid tumor of childhood. High expression of the epidermal growth factor receptor (EGFR) protein has been associated with tumor progression and enhanced tumorigenicity in adult and children gliomas. Nimotuzumab is a humanized antibody that targets the EGFR and has proven efficacy in adult and children gliomas. To provide a new therapeutic option for patients with active, poor prognosis central nervous system (CNS) tumors and to evaluate the feasibility and safety of long-term nimotuzumab therapy in children with diverse CNS tumors, an expanded access program was launched at the Juan Manuel Marquez hospital. Patients were required to be 18 or younger and have one CNS tumor: low-grade glioma (LGG) or high-grade glioma (HGG), brainstem glioma (BSG), ependymoma or primitive neuroectodermal tumor (PNET), and a Lansky or Karnofsky performance status  $\geq 40$ . Treatment consisted of weekly nimotuzumab administered at 150 mg/m<sup>2</sup> for 12 weeks, continuing every 14 days in the absence of severe condition worsening or unacceptable toxicity. Nimotuzumab was administered alone or in combination with radiotherapy, chemotherapy, or both, depending on the tumor type, stage, and previous treatment. Eighty-eight patients, 39 with BSG, 25 with HGG, 9 with progressive LGG, 9 with anaplastic ependymomas, and 6 with other tumor types, including PNET, neuroblastoma, medulloblastoma, and thalamic tumors, were treated with the antibody. The mean number of nimotuzumab doses was 36, from 1 to 108. The most frequent adverse events were mild to moderate skin rash, mucositis, vomiting, seizures, hypothermia, hyperthermia, and paleness. One patient had a grade 3 mucositis, while the other had a grade 3 bleeding on surgery. Sixteen children stopped treatment after at least 2 years with stable disease, partial or complete response. All children were able to maintain the best response achieved on treatment after a 3-year interruption. In summary, this study shows the feasibility of very prolonged administration of nimotuzumab together with the lack of rebound effect after treatment cessation.

**Quantitative image analysis of gamma-H2AX foci induced by ionizing radiation applying open source programs.** González JE, Lee M, Barquinero JF, Valente M, Roch-Lefèvre S, García O. Anal Quant Cytol Histol. 2012 Apr;34(2):66–71.

**Objective** To test a CellProfiler pipeline for automated counting and characterization of gamma-H2AX foci in color images of human cultured cells. **Study design** A431 cells were irradiated and stained for gamma-H2AX foci detection. Sets of color images were analyzed visually, and findings were compared with those using FociCounter and CellProfiler software. **Results** The CellProfiler pipeline includes some proprieties not present in FociCounter, such as the automatic detection of nuclei, the detection of touching nuclei and the rejection of nuclei that touch the border of the image. The time required for manual operation is associated with the number of images analyzed visually or by FociCounter but not for the CellProfiler program. CellProfiler reduced manual operation time and is about 4 times faster than semiautomatic detection using FociCounter and 10 times faster than visual counting. **Conclusion** We conclude that CellProfiler and FociCounter are reliable tools for measuring gamma-H2AX foci. However, CellProfiler overcomes the limitations of the FociCounter program and is able to detect nuclei automatically, saving considerable manual operation.

**Racotumomab: an anti-idiotypic vaccine related to N-glycolyl-containing gangliosides - preclinical and clinical data.** Vázquez AM, Hernández AM, Macías A, Montero E, Gómez DE, Alonso DF, et al. Front Oncol. 2012 Oct 23;2:150. doi: 10.3389/fonc.2012.00150. eCollection 2012.

Neu-glycolyl (NeuGc)-containing gangliosides are attractive targets for immunotherapy with anti-idiotypic mAbs, because these glycolipids are not normal components of the cytoplasmic membrane in humans, but their expression has been demonstrated in several human malignant tumors. Racotumomab is an anti-idiotypic mAb specific to P3 mAb, an antibody which reacts to NeuGc-containing gangliosides, sulfatides, and other antigens expressed in tumors. Preparations containing racotumomab were able to induce a strong anti-metastatic effect in tumor-bearing mice. Different Phase I clinical trials have been conducted in patients with advanced melanoma, breast cancer, and lung cancer. The results of these clinical trials demonstrated the low toxicity and the high immunogenicity of this vaccine. The induced antibodies recognized and directly killed tumor cells expressing NeuGcGM3. A Phase II/III multicenter, controlled, randomized, double blind clinical trial was conducted to evaluate the effect of aluminum hydroxide-precipitated racotumomab vaccine in overall survival in patients with advanced non-small cell lung cancer. The clinical results of this study showed a significant clinical benefit in the patients who were treated with the anti-idiotypic vaccine.

**Radiosensitization induced by the anti-epidermal growth factor receptor monoclonal antibodies cetuximab and nimotuzumab in A431 cells.** González JE, Barquinero JF, Lee M, García O, Casaco A. *Cancer Biol Ther.* 2012 Jan 15;13(2):71–6.

Epidermal growth factor receptors (EGFR) are overexpressed in a wide range of malignancies including head and neck, colon, and breast cancers. It has been identified that carcinomas with high expression levels of EGFR are more resistant to radiotherapy. Therefore, inhibiting nuclear translocation of EGFR to increase the radiosensitivity of malignant cells expressing EGFR offers the potential for increasing the therapeutic index of radiotherapy. The purpose of the present study was to quantify and to compare the radiosensitizing properties of the well-known anti-EGFR antibodies, cetuximab and nimotuzumab in human epidermoid A431 overexpressing EGFR cells. Cells were treated with two concentrations of the antibodies and then irradiated with a single dose of 4 Gy. The results indicated that the two antibodies induced radiosensitization increasing the percentage of dead/dying cells and the yield of  $\gamma$ -H2AX foci 24 h after irradiation. Whereas cetuximab exhibited a significant increase in radiosensitization at the highest concentration, the effects of nimotuzumab were more modest. A correlation between  $\gamma$ -H2AX foci signals and dead/dying cells was observed. The disparity in modulation of radiation-induced DNA damage by the two antibodies could be associated with the level of their respective intrinsic cytotoxic properties. Overall, the findings highlight the potential therapeutic benefit of combination therapy with anti-EGFR antibodies and radiotherapy for relevant carcinomas.

**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;13:299. doi: 10.1186/1471-2407-13-299.

**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.

**Regulation by IFN- $\alpha$ /IFN- $\gamma$  co-formulation (HerberPAG®) of genes involved in interferon-STAT-pathways and apoptosis in U87MG.** Bello C, Vazquez-Blomquist D, Miranda J, Garcia Y, Novoa LI, Palenzuela D, et al. *Curr Top Med Chem.* 2014;14(3):351–8.

Interferons (IFNs) are proteins of the family of cytokines. Their antiproliferative function has been taken into account for several clinical therapies against malignant diseases. In this family, IFNs  $\alpha$  and  $\gamma$  have demonstrated the highest antitumor effects. HerberPAG® is a new co-formulation with IFNs,  $\alpha$ 2b and  $\gamma$ . It has been obtained to increase the antiproliferative effect of individual IFNs and decrease their associated toxicity. Glioblastoma multiforme (GBM) is the most common primary brain tumor and one of the most deadly forms of cancer. The objective of the present work is to obtain insights into the regulation of Interferon-STAT-pathways and apoptosis in U87MG, at the transcriptional level. As a pharmacogenomic strategy we quantified mRNAs levels in vitro by quantitative PCR, using the cell line U87MG as a model. Some of the genes involved in the first steps of IFNs signaling pathways (stat1 and stat3) and apoptosis events (tp53, bax, bcl-2, bad, caspase3 (casp3), caspase8 (casp8) and caspase9 (casp9)) were studied. The detected mRNAs expression pattern for stat1 and stat3 indicates a higher tumor suppressor activity of HerberPAG® compared to individuals IFNs. The up-regulation of tp53, bax, bad, casp3, casp8 and casp9 genes and the down regulation of bcl-2 gene, after the treatment with HerberPAG® show a pro-apoptotic function. HerberPAG® gene-induced profile shows an advantage in relation to IFN  $\alpha$ 2b and  $\gamma$  with a higher stat1 expression and a downregulation of bcl-2 which increases bax:bcl-2 ratio. The regulation of genes involved in IFN-STAT-pathways and apoptosis may be the first evidences to explain the increased antiproliferative properties of this co-formulation.

**Repeated dose (14 days) rat intramuscular toxicology study of Her1 vaccine.** Mancebo A, Casacó A, Sánchez B, González B, Gómez D, León A, et al. Regul Toxicol Pharmacol. 2012 Dec;64(3):425–34.

Our goal was to assess the toxicity of two strengths (200 and 400  $\mu$ g) of HER1 cancer vaccine (Center of Molecular Immunology, Cuba), presented in two different formulations, in Sprague Dawley rats after repeated intramuscular administration (14 days). Four groups (5 animals/sex) were established: Control, Placebo (adjuvant), and two Treated groups receiving a dose representing ten times of human total dose (10 $\times$ ), 28.6 and 57.1  $\mu$ g/kg. Clinical observations, body weight and rectal temperature were measured during the study. Clinical pathology analysis was performed, besides gross necropsy and histological examination of tissues on animals at the end of the assay. The assay ended with a 100% survival. Injection site damage, with the presence of cysts and granulomas, was observed in adjuvant and vaccine treated groups, with most severe cases predominating at higher strength. Administration of Placebo and Her1 vaccine induced increase in polymorphonuclear cells, with relative lymphopenia conditioned by primary neutrophilia. In summary, results suggest that Her1 immunization was capable of inducing an inflammatory effect at the injection site, leading to systemic alterations, more significant at higher strength (400  $\mu$ g, 57.1  $\mu$ g/kg), probably affected by the immunizations' schedule used. The vaccine was shown to be well tolerated without any obvious signs of systemic toxicity, with findings largely attributable to the adjuvant used.

**Repeated dose intramuscular injection of the CIMAvax-EGF vaccine in Sprague Dawley rats induces local and systemic toxicity.** Mancebo A, Casacó A, González B, Ledón N, Sorlozabal J, León A, et al. Vaccine. 2012 May 9;30(22):3329–38.

CIMAvax-EGF consists of a human recombinant epidermal growth factor (EGF), coupled to P64k, a recombinant carrier protein from *N. meningitidis*, and Montanide ISA 51 as adjuvant. The vaccine immunization induces a specific antibody production, inhibiting the EGF/EGF-R interaction through EGF deprivation. The objective of this study was to assess the CIMAvax-EGF toxicity in Sprague Dawley rats after intramuscular administration of repeated doses (6 months) and at the same time to determine if rat is a relevant species for studying CIMAvax-EGF vaccine. Rats were randomly distributed into four groups: control, Montanide ISA 51, treated with 1 $\times$  and 15 $\times$  of human total dose of the antigen. Animals were immunized weekly during 9 weeks, plus 9 immunizations every 14 days. Rats were inspected daily for clinical signs. Body weight, food consumption, and rectal temperature were measured during the administration of doses. Blood samples were collected for hematological, serum biochemical determinations and EGF titles at the beginning, three months and at the end of experimentation. Gross necropsy and histological examination of tissues were performed on animals at the end of the assay. Vaccine provoked the apparition of antibodies against EGF in the rats, demonstrating rat species relevance in these studies. Body weight gain, food and water consumption were not affected. CIMAvax-EGF and Montanide ISA 51 produced local damage at the administration site, showing multiple cysts and granulomas. Both vaccine-treated groups showed neutrophil elevation, besides an AST increase probably related to the damage at the administration site. Rectal temperature was found to be significantly higher in 15 $\times$  treated group after immunizations, probably induced by the inflammatory process at the injection site. In summary, the clinical pathology findings together with the body temperature results, appear to be caused by the inflammatory reaction at the administration site of the vaccine, mainly mediated by the oil-based adjuvant Montanide ISA 51, probably enhanced by the immunological properties of the antigen. This study showed evidences that intramuscular administration during 26 weeks of CIMAvax-EGF at doses up to 15 $\times$  human total dose is well tolerated in rats and it has a clinical importance since this long lasting study in

relevant species allows to treat cancer patients with tumors during long periods with relative weight safety margin.

**Safety, immunogenicity and preliminary efficacy of multiple-site vaccination with an Epidermal Growth Factor (EGF) based cancer vaccine in advanced non small cell lung cancer (NSCLC) patients.**

Rodriguez PC, Neninger E, García B, Popa X, Viada C, Luaces P, et al. J Immune Based Ther Vaccines. 2011 Oct 24;9:7. doi: 10.1186/1476-8518-9-7.

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

**Sensitivity of tumor cells towards CIGB-300 anticancer peptide relies on its nucleolar**

**localization.** Perera Y, Costales HC, Diaz Y, Reyes O, Farina HG, Mendez L, et al. J Pept Sci. 2012 Apr;18(4):215–23.

CIGB-300 is a novel anticancer peptide that impairs the casein kinase 2-mediated phosphorylation by direct binding to the conserved phosphoacceptor site on their substrates. Previous findings indicated that CIGB-300 inhibits tumor cell proliferation in vitro and induces tumor growth delay in vivo in cancer animal models. Interestingly, we had previously demonstrated that the putative oncogene B23/nucleophosmin (NPM) is the major intracellular target for CIGB-300 in a sensitive human lung cancer cell line. However, the ability of this peptide to target B23/NPM in cancer cells with differential CIGB-300 response phenotype remained to be determined. Interestingly, in this work, we evidenced that CIGB-300's antiproliferative activity on tumor cells strongly correlates with its nucleolar localization, the main subcellular localization of the previously identified B23/NPM target. Likewise, using CIGB-300 equipotent doses (concentration that inhibits 50% of proliferation), we demonstrated that this peptide interacts and inhibits B23/NPM phosphorylation in different cancer cell lines as evidenced by in vivo pull-down and metabolic labeling experiments. Moreover, such inhibition was followed by a fast apoptosis on CIGB-300-treated cells and also an impairment of cell cycle progression mainly after 5 h of treatment. Altogether, our data not only validates B23/NPM as a main target for CIGB-300 in cancer cells but also provides the first experimental clues to explain their differential antiproliferative response. Importantly, our findings suggest that further improvements to this cell penetrating peptide-based drug should entail its more efficient intracellular delivery at such subcellular localization.

**[Space analysis of breast and cervix cancer morbimortality. Villa Clara. Cuba. 2004-2009].**

Batista Hernández NE, Alegret Rodriguez M, Antón Fleites O. Rev Esp Salud Publica. 2013 Jan–Feb;87(1):49–57. Spanish.

**Background** Breast and cervix cancers continues being a problem of health, in spite of the existence of prevention and precocious diagnosis programs, and for not existing in our county similar studies, its decide to realized this investigations to identify space and temporal space conglomerate that allow us the analysis of breast and cervix cancer morbimortality in Villa Clara. **Methods** Observational descriptive study to detection space and temporal space of incidence and mortality, using the statistical technique of temporal-space exploration (SatScan programs v .7.01), the sample embraced the entirety of patients diagnosed during the year 2004, to those that were carried out pursuit in the 5 serial years to the diagnosis, until the closing of the year 2009. **Results** The breast cancer showed agreement in the results so much space as temporal-space with a risk of suffering the illness of 1.63 and 1.91 respectively, the municipalities that conformed significant conglomerates, were located toward the center and northeast of the county, headed by Santa Clara the municipality provincial head. The cervix cancer evidenced from the temporary point of view the diagnosis of prospective 100 cases of 68.8 during the months of March to July of the 2004. The study temporal-space



showed a conglomerate of incidence in the municipalities located to the northeast of the county, the women that live in these areas have 3.46 times more risk of presenting this illness than those of the rest of the county. **Conclusions** The presence of significant conglomerates so much was shown space as temporal-space, of the areas of more risk of to make sick for breast and cervix cancers. Not existing of mortality and late diagnosis conglomerates.

**Spatiotemporal analysis of lung cancer incidence and case fatality in Villa Clara Province, Cuba.** Batista NE, Antón OA. MEDICC Rev. 2013 Jul;15(3):16–21

.Introduction Cancer has historically been a main cause of death in Cuba, with lung cancer the number one cause of cancer death in both sexes. Cancer morbidity and mortality rates are the basic measures of cancer impact in the community. Cancer mortality has been one of the major applications of geographic analysis and has made important progress in recent decades thanks to access to mortality statistics and to development and availability of geographic information systems. Cuba does not have a strong tradition of etiologic research using spatial analysis. High levels of lung cancer morbidity and mortality in Villa Clara and growing interest in spatial analysis as an epidemiologic tool motivated this study. Objective To identify spatial and/or spatiotemporal clusters of lung cancer morbidity and case fatality in the province of Villa Clara, and to demonstrate the value of cluster analysis as an epidemiologic tool. Methods Descriptive observational study based on administrative data, using the technique of space-time scan statistics. The study focused on new cases diagnosed in 2004 and case-fatality for those cases through 2009. Variables used were: cases diagnosed, deaths, date of diagnosis, date of death, municipality and Cartesian geocoding for each municipality. Results The study identified significant spatial and spatiotemporal clusters of greater than expected lung cancer incidence (municipalities of Encrucijada, Camajuaní, Cifuentes, Sagua la Grande, Caibarién and Santa Clara) and case fatality (Encrucijada, Camajuaní, Cifuentes, Sagua la Grande, Caibarién, Santa Clara, Placetas and Manicaragua). Conclusions Although the results are not explanatory, the spatial and spatiotemporal patterns of excess lung cancer risk and case-fatality can support hypothesis generation for research and eventual interventions for targeted prevention and management.

**Speaking from experience: today's Cuban women and breast cancer.** Núñez M. MEDICC Rev. 2012 Apr;14(2):10–2.

Over 2200 new cases of breast cancer are diagnosed annually in Cuba, and a decade ago I became one of them. Late in 2000, I underwent breast cancer surgery at the National Oncology and Radiology Institute in the Cuban capital. My experience—both with the disease and as a sociologist at the University of Havana studying gender relations—serves as the basis for the following essay. The article characterizes today's Cuban women, particularly those of us with or at risk of breast cancer, and describes my own and others' responses to our disease. My aim is to provide insights useful to the physicians, nurses, engineers, physicists, technicians, and service and administrative workers in Cuba's health services who interact with us, whose increased awareness will make us feel more deeply understood and respected. In this context, I also reflect on the Cuban media's portrayal of cancer, with recommendations for dismantling the biases of fatalism and even pity often conveyed.

**Specific active immunotherapy with a VEGF vaccine in patients with advanced solid tumors. results of the CENTAURO antigen dose escalation phase I clinical trial.** Gaviñondo JV, Hernández-Bernal F, Ayala-Ávila M, de la Torre AV, de la Torre J, Morera-Díaz Y, et al. Vaccine. 2014 Apr 17;32(19):2241–50.

CIGB-247 is a novel cancer therapeutic vaccine that uses a human VEGF variant molecule as antigen, in combination with a bacterial adjuvant. In mice, CIGB-247 has anti-tumor and anti-metastatic effects. The vaccine induces anti-VEGF blocking antibodies and a cellular response targeting tumor cells producing VEGF, and has proven to be safe in mice, rats, rabbits and non-human primates. Herein we report the results of a Phase I clinical trial (code name CENTAURO) where safety, tolerance, and immunogenicity of CIGB-247 were studied in 30 patients with advanced solid tumors, at three antigen dose levels. Individuals were subcutaneously immunized for 8 consecutive weeks with 50, 100 or 400 µg of antigen, and re-immunized on week twelve. On week sixteen, evaluations of safety, tolerance, clinical status, and immunogenicity (seroconversion for anti-VEGF IgG, serum VEGF/KDR-Fc blocking ability, and gamma-IFN ELISPOT with blood cells stimulated in vitro with mutated VEGF) were done. Surviving patients were eligible for off-trial additional 4-week re-immunizations with 400 µg of antigen. Immunogenicity and clinical status were again studied on weeks 25 and 49. Vaccination was shown to be safe at the three dose levels, with only grade 1-2 adverse events. CIGB-247 was immunogenic and higher numbers of individuals positive to the three immune response tests were seen with increasing antigen dose. Off-protocol long-term vaccination produced no additional adverse events or negative changes in immunogenicity. Eleven patients are still alive, with overall

survivals ranging from 20 to 24 months. Twelve of the thirty patients exhibited objective clinical benefits, and two individuals have complete responses. Most patients with higher survivals are positive in the three immune response tests. In summary, this is the first clinical testing report of a cancer therapeutic vaccine based on a human VEGF related molecule as antigen. The CIGB-247 vaccine is safe, immunogenic, and merits further clinical development.

**Switching on cytotoxicity by a single mutation at the heavy chain variable region of an anti-ganglioside antibody.** Fernández-Marrero Y, Hernández T, Roque-Navarro L, Talavera A, Moreno E, Griñán T, et al. *Mol Immunol.* 2011 Apr;48(8):1059–67.

Gangliosides are sialic acid-containing glycosphingolipids present in the plasma membrane of most mammalian cells. In humans, the expression of the N-glycolylated (Neu5Gc) variant of the sialic acid has been associated with malignant transformation, constituting therefore an attractive target for cancer immunotherapy. P3 monoclonal antibody (mAb) recognizes Neu5Gc-containing gangliosides, as well as sulfatides. Heavy chain CDR3 (H-CDR3) arginine residues have been shown to be crucial for ganglioside recognition, but less important for anti-idiotypic antibody binding. Here, we describe the effect on antibody reactivity of different mutations involving a single H-CDR3 acid residue. Substitution of glutamate 99 (Kabat numbering) by arginine, aspartate or serine residues resulted in no differences in anti-idiotypic binding. However, the first mutation caused increased reactivity with the antigen, including a cytotoxic effect of the antibody on ganglioside-expressing cells previously unseen for the wild type antibody. Another antibody that recognizes N-glycolyl-GM3 ganglioside (GM3(Neu5Gc)), but not other glycolipids, named 14F7, exhibits also an arginine-enriched H-CDR3 and a complement-independent cell death activity. Unlike 14F7 mAb, the cytotoxicity of the P3 E(99)→R mutant antibody did not exclusively depend on ganglioside expression on tumor cells.

**Synchronous bilateral breast cancer in a male.** Rubio Hernández MC, Díaz Prado YI, Pérez SR, Díaz RR, Aleaga ZG. *Ecanermedicalscience.* 2013 Dec 4;7:377. doi: 10.3332/ecancer.2013.377. eCollection 2013.

Male breast cancer, which represents only 1% of all breast cancers, is occasionally associated with a family history of breast cancer. Sporadic male breast cancers presenting with another primary breast cancer are extremely rare. In this article, we report on a 70-year-old male patient with bilateral multifocal and synchronous breast cancer and without a family history of breast cancer.

**The anti-cancer agent guttiferone-A permeabilizes mitochondrial membrane: ensuing energetic and oxidative stress implications.** Pardo-Andreu GL, Nuñez-Figueroa Y, Tudella VG, Cuesta-Rubio O, Rodrigues FP, Pestana CR, et al. *Toxicol Appl Pharmacol.* 2011 Jun 15;253(3):282–9.

Guttiferone-A (GA) is a natural occurring polyisoprenylated benzophenone with cytotoxic action in vitro and anti-tumor action in rodent models. We addressed a potential involvement of mitochondria in GA toxicity (1-25 µM) toward cancer cells by employing both hepatic carcinoma (HepG2) cells and succinate-energized mitochondria, isolated from rat liver. In HepG2 cells GA decreased viability, dissipated mitochondrial membrane potential, depleted ATP and increased reactive oxygen species (ROS) levels. In isolated rat-liver mitochondria GA promoted membrane fluidity increase, cyclosporine A/EGTA-insensitive membrane permeabilization, uncoupling (membrane potential dissipation/state 4 respiration rate increase), Ca<sup>2+</sup> efflux, ATP depletion, NAD(P)H depletion/oxidation and ROS levels increase. All effects in cells, except mitochondrial membrane potential dissipation, as well as NADPH depletion/oxidation and permeabilization in isolated mitochondria, were partly prevented by the a NAD(P)H regenerating substrate isocitrate. The results suggest the following sequence of events: 1) GA interaction with mitochondrial membrane promoting its permeabilization; 2) mitochondrial membrane potential dissipation; 3) NAD(P)H oxidation/depletion due to inability of membrane potential-sensitive NADP<sup>+</sup> transhydrogenase of sustaining its reduced state; 4) ROS accumulation inside mitochondria and cells; 5) additional mitochondrial membrane permeabilization due to ROS; and 6) ATP depletion. These GA actions are potentially implicated in the well-documented anti-cancer property of GA/structure related compounds.

**The Antitumor Peptide CIGB-552 Increases COMMD1 and Inhibits Growth of Human Lung Cancer Cells.** Fernández Massó JR, Oliva Argüelles B, Tejada Y, Astrada S, Garay H, Reyes O, et al. *J Amino Acids.* 2013;2013:251398. doi: 10.1155/2013/251398. Epub 2013 Jan 16.

We have demonstrated that the peptide L-2 designed from an alanine scanning of the Limulus-derived LALF32-51 region is a potential candidate for the anticancer therapy and its cell-penetrating capacity is an

associated useful property. By the modification in the primary structure of L-2, a second-generation peptide (CIGB-552) was developed. However, the molecular mechanism underlying its cytotoxic activity remains partially unknown. In this study, it was shown that CIGB-552 increases the levels of COMMD1, a protein involved in copper homeostasis, sodium transport, and the NF- $\kappa$ B signaling pathway. We found that CIGB-552 induces ubiquitination of RelA and inhibits the antiapoptotic activity regulated by NF- $\kappa$ B, whereas the knockdown of COMMD1 blocks this effect. We also found that CIGB-552 decreases the antioxidant capacity and induces the peroxidation of proteins and lipids in the tumor cells. Altogether, this study provides new insights into the mechanism of action of the peptide CIGB-552, which could be relevant in the design of future anticancer therapies.

**Therapeutic approaches to target cancer stem cells.** Diaz A, Leon K. *Cancers (Basel)*. 2011 Aug 15;3(3):3331–52.

The clinical relevance of cancer stem cells (CSC) remains a major challenge for current cancer therapies, but preliminary findings indicate that specific targeting may be possible. Recent studies have shown that these tumor subpopulations promote tumor angiogenesis through the increased production of VEGF, whereas the VEGF neutralizing antibody bevacizumab specifically inhibits CSC growth. Moreover, nimotuzumab, a monoclonal antibody against the epidermal growth factor receptor (EGFR) with a potent antiangiogenic activity, has been shown by our group to reduce the frequency of CSC-like subpopulations in mouse models of brain tumors when combined with ionizing radiation. These studies and subsequent reports from other groups support the relevance of approaches based on molecular-targeted therapies to selectively attack CSC. This review discusses the relevance of targeting both the EGFR and angiogenic pathways as valid approaches to this aim. We discuss the relevance of identifying better molecular markers to develop drug screening strategies that selectively target CSC.

**Therapeutic vaccination with an EGF-based vaccine in lung cancer: a step in the transition to a chronic disease.** Rodriguez G, Gonzalez G, Crombet T, Lage A. *Expert Rev Respir Med*. 2011 Jun;5(3):337–42.

The trend of increased survival in advanced tumors suggests the possibility of the transformation of cancer into a chronic disease. That goal will require therapeutic weapons with low toxicity that can be used chronically. Here we summarize the development of a therapeutic vaccine consisting in recombinant EGF chemically linked to a protein from *Neisseria meningitidis*. In mice, the vaccine elicited antibodies to self-EGF and had anti-tumor activity. Clinical trials have shown that the vaccine is also immunogenic and well tolerated in humans. The vaccination produced a decrease in plasma EGF concentration. Advanced lung cancer patients eliciting high antibody titers of EGF had better survival. The vaccine can be used long term and integrated with other treatment modalities.

**The regulatory framework for similar biotherapeutic products in Cuba.** Hechavarría Núñez Y, Pérez Massipe RO, Orta Hernández SD, Muñoz LM, Jacobo Casanueva OL, Pérez Rodríguez V, et al. *Biologicals*. 2011 Sep;39(5):317–20.

Biopharmaceuticals make up a significant proportion of medicinal products used for the treatment of diseases such as cancer, arthritis, cardiac dysfunctions and AIDS. Access to therapies based on the use of these products has been limited as a result of the high marketing costs. Cuba has a biopharmaceutical industry with great potential for innovation, capable of developing new products and to produce others, like the biosimilars destined to fulfill the needs of its National Health System. The Center for State Control on the Quality of Drugs (CECMED) the Cuban NRA, is facing the challenge of regulating the approval of biosimilar products manufactured locally. Consequently, CECMED has issued a position paper establishing the basic principles for regulation of these products and a specific guideline on this was elaborated.

**Towards the final BSA modeling for the accelerator-driven BNCT facility at INFN LNL.** Ceballos C, Esposito J, Agosteo S, Colautti P, Conte V, Moro D, et al. *Appl Radiat Isot*. 2011 Dec;69(12):1660–3.

Some remarkable advances have been made in the last years on the SPES-BNCT project of the Istituto Nazionale di Fisica Nucleare (INFN) towards the development of the accelerator-driven thermal neutron beam facility at the Legnaro National Laboratories (LNL), aimed at the BNCT experimental treatment of extended skin melanoma. The compact neutron source will be produced via the  $(^9\text{Be}(p,xn))$  reactions using the 5 MeV, 30 mA beam driven by the RFQ accelerator, whose modules construction has been recently completed, into a thick beryllium target prototype already available. The Beam Shaping Assembly (BSA) final modeling, using

both neutron converter and the new, detailed, Be(p,xn) neutron yield spectra at 5 MeV energy recently measured at the CN Van de Graaff accelerator at LNL, is summarized here.

**Treatment of children with high grade glioma with nimotuzumab: a 5-year institutional experience.** Cabanas R, Saurez G, Rios M, Alert J, Reyes A, Valdes J, et al. MAbs. 2013 Mar-Apr;5(2):202–7.

Brain tumors are a major cause of cancer-related mortality in children. Overexpression of epidermal growth factor receptor (EGFR) is detected in pediatric brain tumors and receptor density appears to increase with tumor grading. Nimotuzumab is an IgG1 antibody that targets EGFR. Twenty-three children with high-grade glioma (HGG) were enrolled in an expanded access program in which nimotuzumab was administered alone or with radio-chemotherapy. The mean number of doses was 39. Nimotuzumab was well-tolerated and treatment with the antibody yielded a survival benefit: median survival time was 32.66 mo and the 2-y survival rate was 54.2%. This study demonstrated the feasibility of prolonged administration of nimotuzumab and showed preliminary evidence of clinical benefit in HGG patients with poor prognosis.

**Treatment of malignant, non-resectable, epithelial origin esophageal tumours with the humanized anti-epidermal growth factor antibody nimotuzumab combined with radiation therapy and chemotherapy.** Ramos-Suzarte M, Lorenzo-Luaces P, Lazo NG, Perez ML, Soriano JL, Gonzalez CE, et al. Cancer Biol Ther. 2012 Jun;13(8):600–5.

**Background** Over-expression of epidermal growth factor receptor in esophageal cancer is associated with poor prognosis. The present study was conducted to evaluate safety and preliminary efficacy of nimotuzumab, a humanized anti-EGFR antibody in combination with radiation and chemotherapy in advanced esophageal tumours. Patients and **Methods** A Phase II clinical trial was conducted, where patients received cisplatin, 5-fluorouracil, and radiotherapy, either alone or combined with six weekly infusions of nimotuzumab at the dose of 200 mg. Safety was the primary endpoint. The antitumoral objective response rate was the secondary endpoint. Epidermal growth factor receptor expression, KRAS mutation status and anti-idiotypic response were also evaluated. **Results** Sixty-three patients were included in the study. Thirty patients were entered into the control group, and thirty-three patients received the treatment with nimotuzumab. The antibody was very well tolerated. Objective response rate was 47.8 % (nimotuzumab group) and 15.4 % (control group). Disease control rate was 60.9 % (nimotuzumab group) and 26.9 % (control group). Response and disease control rate were higher in patients with EGFR overexpressing tumors. **Conclusions** Nimotuzumab plus chemoradiotherapy was safe and provided statistically significant objective response. A Phase III in patients with similar characteristics will be launched.

**Trends in digestive cancer mortality, Cuba 1987-2008.** Abreu MR, Vilar E, Arús ER, Mejia JM, Martínez Y, Yasells AA. Eur J Public Health. 2013 Feb;23(1):164–70.

**Background** Gastrointestinal malignancies are among the most common cancers suffered by Cubans. The purpose of our study is to analyse the evolution of digestive cancer mortality in Cuba. **Methods** Mortality data for this study were obtained from the National Medical Records and Health Statistic Bureau. Trends (1987-2008) in age-standardized cancer mortality were described using joinpoint regression. **Results** In the data set of digestive cancer mortality, in the period 1987-2008, colorectal/anal cancer was the most frequent cause of cancer mortality in males and females. In men, a rise in mortality was observed for cancer of the oesophagus between 2001 and 2008, and pancreatic cancer showed a slight mortality rise for the period 1987-2008. In women, colorectal/anal cancer increased from 1989 to 2001. A mortality increase was observed for oesophageal cancer in the period 2005-08. The result of the joinpoint analysis for the age group of 35-64 years was consistent with those for overall mortality. **Conclusions** The trend in mortality from digestive cancer in Cuba shows differences depending on sex, age and type of tumour. The Cuban health system has seen improvements in diagnostic systems, which has contributed even better diagnoses of digestive diseases.

**Vaccination with a mutated variant of human Vascular Endothelial Growth Factor (VEGF) blocks VEGF-induced retinal neovascularization in a rabbit experimental model.** Morera Y, González R, Lamdan H, Pérez L, González Y, Agüero J, et al. Exp Eye Res. 2014 May;122:102–9.

Vascular Endothelial Growth Factor (VEGF) is a key driver of the neovascularization and vascular permeability that leads to the loss of visual acuity of eye diseases like wet age-related macular degeneration, diabetic macular edema, and retinopathy of premature. Among the several anti-VEGF therapies under investigation for the treatment of neovascular eye diseases, our group has developed the vaccine candidate CIGB-247-V that uses a mutated form of human VEGF as antigen. In this work we evaluated if the vaccine could prevent or

attenuate VEGF-induced retinal neovascularization in the course of a rabbit eye neovascularization model, based on direct intravitreal injection of human VEGF. Our experimental findings have shown that anti-VEGF IgG antibodies induced by the vaccine were available in the retina blood circulation, and could neutralize in situ the neovascularization effect of VEGF. CIGB-247-V vaccination proved to effectively reduce retinal neovascularization caused by intravitreal VEGF injection. Altogether, these results open the way for human studies of the vaccine in neovascular eye syndromes, and inform on the potential mechanisms involved in its effect.

[Very small size proteoliposomes abrogate cross-presentation of tumor antigens by myeloid-derived suppressor cells and induce their differentiation to dendritic cells](#). Fernández A, Oliver L, Alvarez R, Hernández A, Raymond J, Fernández LE, et al. *J Immunother Cancer*. 2014 Mar 11;2:5. doi: 10.1186/2051-1426-2-5. eCollection 2014.

**Background** Myeloid-derived suppressor cells (MDSCs) are among the major obstacles that adjuvants for cancer vaccines have to overcome. These cells cross-present tumor-associated antigens (TAA) to naive T lymphocytes with a tolerogenic outcome. Very Small Size Proteoliposomes (VSSP) is used as adjuvant by four therapeutic cancer vaccines currently in Phase I and II clinical trials. We previously found that VSSP reduces the suppressive function of MDSCs, then activating antigen-specific CTL responses in tumor-bearing (TB) mice, with the consequent reduction of tumor growth. However the mechanistic explanation for the immunomodulatory effect of this adjuvant in TB hosts has not been addressed before. **Methods** TB mice were inoculated with VSSP and MDSCs isolated and characterized by their expression of Arg1 and Nos2 genes by RT-PCR. The effect of VSSP on antigen cross-presentation by MDSCs, regulatory T cells (Tregs) expansion and MDSCs differentiation towards dendritic cells (DCs) was analyzed by FACS. Student's t test or ANOVA and Tukey's tests were used for statistical analyses. **Results** After inoculating VSSP into TB mice, a significant reduction of Arg1 and Nos2 gene expression was observed in recovered MDSCs. Concurrently the ability of these cells to induce down-regulation of CD3 $\zeta$  chain on T cells was lost. Likewise in mice inoculated with the adjuvant lower percentages of Tregs were detected. In vitro, VSSP treatment was enough to differentiate MDSCs into phenotypically mature DCs, eliminating the former suppressive effect. Noteworthy, in vivo administration of VSSP to OVA-expressing (EG.7) TB mice abrogated this model antigen cross-presentation by splenic MDSCs. Similar results were obtained even when OVA antigen was administered into these TB mice formulated in VSSP. On the contrary, immunization with the same protein in polyI:C did not change the percentage of MDSCs expressing SIINFEKL/H-2K(b) complexes, whereas a concomitant injection of VSSP aborted the limitations of polyI:C in this setting. **Conclusions** Altogether, these results indicate that VSSP has the peculiar capacity of inhibiting TAA cross-presentation and certain suppressive mechanisms on MDSCs which in turn, combined with the ability to induce differentiation of these cells into antigen-presenting cells (APCs), sustains this adjuvant as an ideal immunomodulator for cancer immunotherapy.

[Very small size proteoliposomes \(VSSP\) and Montanide combination enhance the humoral immune response in a GnRH based vaccine directed to prostate cancer](#). Aguilar FF, Barranco JJ, Fuentes EB, Aguilera LC, Sáez YL, Santana MD, Vázquez EP, Baker RB, et al. *Vaccine*. 2012 Oct 12;30(46):6595–9.

Very small size proteoliposomes (VSSP) constitute a complex of very small size proteoliposomes that includes proteins, lipids, CpG and gangliosides tumor-associated that provides a potential target for cancer immunotherapy. This compound has been described to stimulate the humoral and cellular response, dendritic cells (DC) activation and differentiation of T-helper cells, specially, in immunocompromised patients with cancer status. This work deals with the stimulating capacity of the VSSP to reach a humoral response when they are used as a component in a peptidic vaccine based on the gonadotrophin releasing hormone (GnRH). This study was carried out in male Copenhagen rats, which were immunized with 750 $\mu$ g of the GnRH mimetic peptide (GnRHm1-TT) with or without the VSSP. The mixtures were always emulsified with the oil adjuvant Montanide ISA 51. The anti GnRH seroconversion analysis revealed that the group immunized with the peptide GnRHm1-TT/VSSP developed a strong anti GnRH seroconversion. These antibody levels proved to be significant superior to those reached by the use of the GnRHm1-TT peptide solely emulsified in Montanide. Post-mortem analysis on the Testosterone ablation target organs (prostate and testicles) yielded a sudden decrease in their size and weight in respect to the control group. On the other hand, the group submitted to the use of GnRHm1-TT/VSSP, showed a significant difference in the reduction of these target organs in comparison with the group only immunized with GnRHm1-TT adjuvated in Montanide ISA 51. These values turned to be of  $p=0.023$  and  $p=0.009$  in the prostate and testicles respectively. These findings foreground the VSSP as a useful immunopotentiator to be used as part of a GnRH based vaccine to treat prostate cancer.

**Very small size proteoliposomes derived from *Neisseria meningitidis*: an effective adjuvant for antigen-specific cytotoxic T lymphocyte response stimulation under leukopenic conditions.** Oliver L, Fernández A, Raymond J, López-Requena A, Fernández LE, Mesa C. Vaccine. 2012 Apr 19;30(19):2963–72.

Leukopenia is a severe condition resulting from both pathological processes and some treatments, like chemotherapy in cancer patients. However, the activation of the patient immune system is required for the success of immunotherapeutic strategies, as cancer vaccines. In this regard, leukopenia constitutes a major hurdle to overcome, mainly due to the impairment of cytotoxic T lymphocyte (CTL) responses. Adjuvants are basic components of vaccine formulations, which might be useful to stimulate immunity under this immunosuppressed condition. To this aim, we tested the capacity of a novel nanoparticulated complex, very small size proteoliposomes (VSSP), to promote CTL even in a leukopenic scenario. Noteworthy, we observed that a VSSP-based OVA vaccine induced a normal antigen-specific CTL response in mice rendered leukopenia by the administration of high doses of the chemotherapeutic agent cyclophosphamide (CY), while under the same conditions the OVA antigen formulated in the TLR-3 agonist polyinosinic-polycytidylic acid (P(I:C)) was ineffective. Moreover, an appropriate combination of VSSP with the P(I:C) vaccine was able to restore the CD8(+) T cell effector function in leukopenic mice. VSSP induced not only a faster repopulation of immune cells in CY-receiving animals, but also enhanced the recovery of memory T lymphocytes and myeloid dendritic cells (DCs) while simultaneously abrogated the immunosuppressive capacity of myeloid-derived suppressor cells (MDSCs). Our results suggest that VSSP could be a particularly suitable immunomodulator to be used in CTL-promoting active immunotherapy strategies operating in severe immune compromised scenarios.

**Yttrium-90 —current status, expected availability and applications of a high beta energy emitter.** Montaña RL, González IH, Ramirez AA, Garaboldi L, Chinol M. Curr Radiopharm. 2012 Jul;5(3):253–63.

Yttrium-90 ( $^{90}\text{Y}$ ,  $T(1/2)$  64.14 h) is a key example of a high beta energy-emitting radionuclide which is available from the strontium-90 ( $^{90}\text{Sr}/^{90}\text{Y}$ ) radionuclide generator system. Clinical uses of  $^{90}\text{Y}$ -labeled radiopharmaceutical agents have been pursued for many years and many applications have proven to be clinically effective. These most notably include the application of  $^{90}\text{Y}$ -labeled antibodies for a variety of applications such as for effective treatment of non-Hodgkin's lymphoma. One of the major advantages for use of  $^{90}\text{Y}$  is ready availability from the very long-lived  $^{90}\text{Sr}$  parent ( $T(1/2)$  28.78 y). Because of the importance of maintaining generator performance and minimizing parent breakthrough, this paper describes development, use and quality control of both high capacity cation adsorption-type and electrochemical generator systems. In addition, the preparation and targeting to tumors in mice of DOTA-conjugated Nimotuzumab (h-R3) antibody which recognizes the external domain of the EPFR antibody radiolabeled with  $^{90}\text{Y}$  obtained from the electrochemical generator is also described. As a key example for clinical applications of  $^{90}\text{Y}$ , the use of  $^{90}\text{Y}$ -labeled biotin for intra-operative pre-targeting for radionuclide therapy (IART®) of breast cancer is also described.