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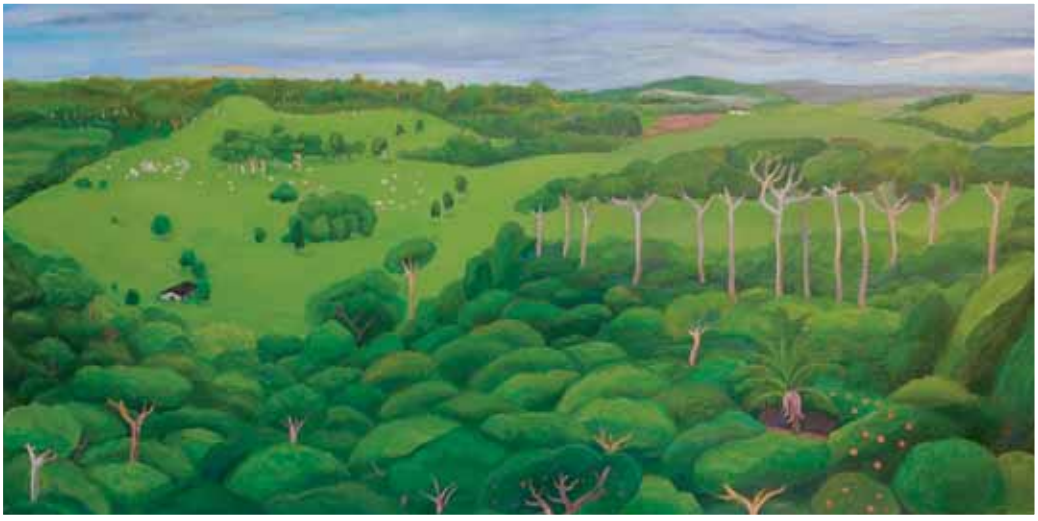
BUENOS AIRES VOL. 78 Supl. III - 2018



MEDICINA

Volumen 78, Supl. III, págs. 1-273

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COMITÉ DE REDACCIÓN

La Tapa (Ver p xx)
Los palos rosas, 2015
Daniela Kantor

MEDICINA (Buenos Aires) – Revista bimestral – ISSN 0025-7680 (Impresa) – ISSN 1669-9106 (En línea)

REVISTA BIMESTRAL
Registro de la Propiedad Intelectual N° 5350968
Personería Jurídica N° C-7497
Publicación de la Fundación Revista Medicina (Buenos Aires)
Propietario de la publicación: Fundación Revista Medicina
Queda hecho el depósito que establece la Ley 11723

Publicada con el apoyo del Ministerio de Ciencia, Tecnología e Innovación Productiva.
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Incluida en el Núcleo Básico de Revistas Científicas Argentinas del CONICET.

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Tel. 5287-3827 Int. 73919 y 4523-6619
e-mail: revmedbuenosaires@gmail.com – http//: www.medicinabuenosaires.com

Vol. 78, Supl.III, Noviembre 2018

Edición realizada por
Diseño y Diagramación: Andrés Esteban Zapata - aez.sgi@gmail.com - 11 5509 2767
Impreso en PQC - Berón de Astrada 2064 - C.A.B.A. - 4919 1702

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BUENOS AIRES, VOL 78 Supl. III - 2018

REUNIÓN CONJUNTA SAIC SAI SAFIS 2018

LXIII REUNIÓN ANUAL DE LA

SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

LXVI REUNIÓN ANUAL DE LA

SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

REUNIÓN ANUAL DE LA

SOCIEDAD ARGENTINA DE FISIOLOGÍA (SAFIS)

CON LA PARTICIPACIÓN DE

SOCIEDAD ARGENTINA DE VIROLOGÍA (SAV)

ASOCIACIÓN ARGENTINA DE NANOMEDICINAS (NANOMED-ar)

14-17 de noviembre de 2018
Hotel 13 de Julio – Mar del Plata

EDITORES RESPONSABLES

Claudia Pérez Leirós
Pablo Baldi
Alberto Crottogini

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BUENOS AIRES, VOL 78 Supl. III - 2018

JOINT MEETING SAIC SAI SAFIS 2018

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ANNUAL MEETING OF

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WITH THE PARTICIPATION OF

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ASOCIACIÓN ARGENTINA DE NANOMEDICINAS (NANOMED-ar)

November 14-17, 2018
Hotel 13 de Julio – Mar del Plata

RESPONSIBLE EDITORS

Claudia Pérez Leirós
Pablo Baldi
Alberto Crottogini

Ruben Marrero[1], Cristina Seki[3], Guido König[1, 2]
[1]*Instituto Biotecnología, INTA* , [2]*CONICET* , [3]*Centro de Virología Animal (CEVAN)*

Foot-and-mouth disease (FMD) is one of the major animal diseases of agronomic relevance and it is caused by the FMD virus (FMDV). The FMDV control is mainly exerted by neutralizing antibodies that recognize functional-relevant viral regions (antigens; Ag). Few FMDV-Ab complexes had been studied by crystal diffraction methods and characterized at a quasi-atomic level, but there are no investigations that extrapolate this information to analyze unknown structures from different FMDV strains antibodies.
Currently, we have evaluated the effectiveness of eight computational programs to model the interaction between serotype C FMDV site A Ag and two different monoclonal antibodies (mAbs). Specifically, we model all possible point mutations through the amino acid residues of a viral antigenic peptide and generate a computational profile of the Ag/mAb interaction energy. These results were compared with the interaction data, obtained previously by ELISA. The best correlation was obtained with the program FoldX showing a Pearson correlation (r) of 0.7, with a high significant level of confidence (simple Mantel test).
Secondly, we model multiple amino acid mutations representative of FMDV outbreaks interacting with the referred mAbs. The emerging data from this experiments showed a coefficient R2 of 0,53 for the FoldX program which also yields the better AUC value (0.91) in a ROC analysis.
Finally, we cloned the coding sequence of further two mAbs that also recognize and neutralize site A epitopes from a serotype A virus. Then, we implemented a protocol for modeling their molecular structure as well as their interaction with viral Ag, assisted by the information obtained with peptide ELISA test mutational profiles. By means of those results, we developed a differential and functional epitope fine map for those mAbs.

480. (684) **TIM-3 INCREASE DURING ANTI PD-1 TREATMENT IS ASSOCIATED WITH DISEASE PROGRESSION IN PATIENTS WITH NON-SMALL CELL LUNG CANCER AND RENAL CARCINOMA**

Estefanía Paula Juliá, Pablo Mando, Manglio Miguel Rizzo, Florencia Tsou, Romina Luca, Alicia Inés Bravo, Walter Astorino, Jose Mordoh, Claudio Martín, Estrella Levy
Centro de Investigaciones Oncológicas-Fundación Cáncer, Ciudad Autonoma de Buenos Aires, Argentina.

Cancer immunotherapies targeting PD-1/PD-L1 axis have shown efficacy in a wide range of cancers. However, not all patients benefit from treatment. In a cohort of 18 non-small cell lung cancer (NSCLC) and 7 renal carcinoma (RC) patients, we assessed immune cell populations and soluble mediators in peripheral blood before (PRE) and after (POST) 8-12 weeks of therapy with anti-PD-1 mAbs Pembrolizumab or Nivolumab. The aim was to identify potential biomarkers of response.
We used an automated hematology analyzer to study white blood cell counts and flow cytometry to analyze lymphocyte subpopulations (CD4, CD8 and regulatory T cells and NK cells) and markers of activation/differentiation on T cells. Plasmatic C-Reactive Protein (CRP) and cytokine concentration were measured using CRP assay and Cytometric Bead Array, respectively.
12 patients presented stable disease or response (SD-R) while 9 patients progressed (PD). Response was not evaluable in 4 patients. No differences were observed in any of the markers analyzed in pre-treatment samples between PD and SD-R patients. So we compared the variation (POST minus PRE median values) of each marker between both response groups. Patients with PD, in contrast to SD-R, presented an increase in the percentage of TIM-3+ within CD4 (median variation [IQR]: +2.8% [+1.7–+4.1] vs -1.6% [-2.9– -1.2], p=0.0018) and CD8 (+5.5% [+4.0–+6.5] vs -1.9% [-2.3– -0.38], p=0.0009) T cells. PFS analysis showed that increase of TIM-3 expressing cells was deleterious for survival (CD4 p=0.001; CD8 p=0.002). Evaluation of soluble mediators' variation showed significant differences between PD and SD-R patients in CPR (+9.9mg/l vs -5.9mg/l, p=0.03) and IL-8 (+8.8pg/ml vs -3.1pg/ml, p=0.015) plasma levels.
TIM-3 expression in TILs has been previously described as a resistance mechanism to anti-PD-1 therapy. The evaluation of TIM-3 in peripheral blood lymphocytes may be a more accessible and useful tool to monitor progression to this therapy.

481. (699) **AUTOLOGOUS T CELL ACTIVATION FOSTERS ABT-199 RESISTANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND SELECTS MALIGNANT CELLS WITH AN AGGRESSIVE PHENOTYPE.**

Esteban Enrique Elías, Ana Colado, Maricef Vergara Rubio, Gregorio Cordini, Denise Risnik, Horacio Fernandez Grecco, Fernando Raimundo Bezares, María del Rosario Custidiano, Julio César Sánchez Avalos, Ángeles Vicente, Gonzalo Martín Garate, Mercedes Borge, Mirta Giordano, Romina Gamberale
IMEX-CONICET, Academia Nacional de Medicina

BCR signaling and activated T cells from the microenvironment favor malignant cell activation, proliferation and survival in CLL. ABT-199, a specific BCL-2 inhibitor, is highly cytotoxic against unstimulated CLL cells. We reported that T cell activation induces ABT-199 resistance in CLL cells (Elías-Haematologica-2018). To further characterize resistant CLL cells, PBMC from CLL patients were cultured for 48hs without (control) or with anti-CD3 (aCD3) to activate T cells, and then ABT-199 was added to the cultures. Leukemic cell survival, activation and proliferation capacity were evaluated by flow cytometry. While control CLL cells treated with ABT-199 showed more than 93% of cell death after 48hs of ABT-199 treatment, CLL cells from aCD3 cultures are still alive with ABT-199 at 120hs (%CD19+ viable cells: 63±8 vs 34±7 for aCD3 vs aCD3+ABT-199) and show similar levels of the activation marker CD86 (n=9). Interestingly, CLL cells in aCD3+ABT-199 cultures showed increased size (n=9, $p<0.01$) and higher Ki67 expression (n=6, $p<0.05$) compared to aCD3 cultures. To overcome ABT-199 resistance we previously used the BCR kinase inhibitor (BCR-KI) GS-9973 (Elias-Haematologica-2018). Here we showed that GS-9973 did not directly affect leukemic cell survival or modify ABT-199-induced cell death (n=10), corroborating that its effects were through impairment of T cell activation (Colado-Cancer. Immunol.Immunother-2017). Finally, we reported that GS-9973 reduces phagocytosis of rituximab-coated CLL cells (Colado-Cancer. Immunol.Immunother-2017), while ABT-199 enhances it (Elias-Haematologica-2018). We here found that obinutuzumab-coated CLL cells phagocytosis was reduced by GS-9973 (n=5, $p<0.05$) and ABT-199 seemed to enhance it (%phagocytosis: 40±8 vs 68±9 for Obinutuzumab+GS-9973 vs Obinutuzumab+GS-9973+ABT-199; n=5), although this difference is not statistically significant yet. Moreover, ABT-199 did not affect CD107a degranulation by NK cells induced by anti-CD20-coated CLL cells (n=5). Our results confirm that activated leukemic cells from the microenvironment might not be properly targeted by ABT-199 monotherapy and encourage its combination with BCR-KI and anti-CD20 antibodies.

ONCOLOGÍA / ONCOLOGY ORAL SESSION 3

482. (149) **ANTITUMORAL AND IMMUNOMODULATORY ROLE OF HISTAMINE IN BREAST CANCER**

Melisa Nicoud, Helena A Sterle, Noelia Massari, Mónica Táquez Delgado, Karina Formoso, Verónica Herrero Ducloux, Diego Martinel Lamas, Vanina Medina
Investigaciones Biomédicas (BIOMED) UCA-CONICET

It is well known that histamine is a key regulator of immune cell functions and it also modulates cancer cell proliferation. The aim of this work was to investigate the effect of histamine and its H4 receptor (H4R) agonist (JNJ28610244) on tumour growth and in the immune tumour microenvironment as a whole, in a triple negative breast cancer (TNBC) syngeneic model developed in immunocompetent mice. Tumours of the TNBC cell line 4T1 were established in Balb/c mice. Treatments employed: histamine (1 or 5 mg/kg) and JNJ28610244 (1 or 5 mg kg). Results show that histamine treatment (5 mg/kg) reduces tumour growth more effectively than JNJ28610244. Histamine but not the agonist increases tumour apoptosis and it reduces

the number of intratumoural vessels. Histamine also reduces immunosuppression through the modulation of the tumour microenvironment, as it increases the tumour secretion of IFN gamma and reduces the number of T regulatory (Treg) lymphocytes in lymph nodes and spleen.
A lower concentration (1 mg/kg) of JNJ28610244 reduces tumour size while no immunomodulatory effects are observed in the immune cell subsets studied. In contrast, a higher concentration (5 mg/kg) is not able to decrease tumour growth probably because of the immunosuppressive effect produced in the tumour microenvironment, showing increased levels of interleukin (IL)-10 and decreased levels of IFNγ in tumours and increased infiltrating Treg cells in tumour draining lymph nodes. These results highlight the critical interplay between tumour cells and host immune response that determine the clinical therapeutic outcomes and suggest that histamine is a key pleiotropic mediator with therapeutic benefits in TNBC.

483. (312) **MULTIVARIATE ANALYSIS OF IMMUNE CELLS POPULATIONS INVOLVED IN TUMOR GROWTH**
Antonela Del Giúdice, María Celeste Capitani, Matías Fusini, Leandro Mainetti, O. Graciela Scharovsky, Ricardo Di Masso, María José Rico, Viviana R. Rozados
Facultad de Ciencias Médicas - Universidad Nacional de Rosario

M-406 mammary adenocarcinoma appeared in an inbred CBi mouse. CBi- mice were artificially selected by body conformation from CBi. When CBi- mice are s.c. challenged with M-406, 100% of the tumors are rejected; conversely, tumor grows exponentially in CBi. To explain the participation of the immune system in this behavior, CBi and CBi- females (N=9), were inoculated with M-406, blood samples were taken on days 0, 7 and 14, and CD4+, CD8+, Treg and Th17 cells were quantified (flow cytometry). Day 0- CD4+: CBi<CBi- (P=0.0133), CD8+: CBi>CBi- (P=0.0041), Treg: CBi>CBi- (P=0.0003) and Th17: CBi<CBi- (P=0.0111) (Student's t). The analysis with the multivariate technique of principal components, generated two components (PC). CBi: PC1- day 0 (mean ± SEM,0.4 ± 3.18), day 7 (-2.8 ± 5.14), day 14 (2.4 ± 2.14) explained 79.27% of the variance and was negatively correlated with CD4+ (P<0.0001); PC2- day 0 (-0.6 ± 1.57), day 7 (-3.4 ± 1.09), day 14 (3.9 ± 1.85), explained 20.23% of the variance and was negatively correlated with CD8+ (P<0.0001). No associations were observed for Treg and Th17. CBi-: PC1- day 0 (15.3 ± 4.10), day 7 (-21.5± 5.30), day 14 (9.5 ± 3.5) explained 98.25% of the variance and was positive and negatively correlated with CD4+ (P<0.001) and Th17 (P<0.0001), respectively; PC2- day 0 (0.4 ± 0.95), day 7 (-0.5 ± 0.62), day 14 (0.8 ± 0.97) explained 1.57% of the variance and was negatively correlated with CD8+ (P<0.0001). No association was observed for Treg. 1) Treg cells were not associated with tumor growth/rejection. 2) The antagonistic behavior observed in both lines of mice challenged with M-406 could be mainly explained by the different evolution of CD4+ and Th17 cells during tumor growth (PC1) 3) PC2 (CD8) explains the variance found in CBi.

484. (363) **TNFA BLOCKADE IMPROVES ANTITUMOR INNATE IMMUNE RESPONSE AND OVERCOMES TRASTUZUMAB-RESISTANCE IN HER2+ BREAST CANCER**
Sofia Bruni, Mara De Martino, Maria Florencia Mercogliano, Cecilia Proietti, Isabel Frahm, Patricia Elizalde, Roxana Schillaci
Instituto de Biología y Medicina Experimental (IBYME - CONICET)

HER2 positive (HER2+) is a subtype that affects 13-20% of breast cancer (BC) patients. They receive trastuzumab (T), an anti-HER2 monoclonal antibody, but 40-60% of them relapse. Therefore, new strategies to overcome trastuzumab resistance are needed. We recently demonstrated a novel tumor immune evasion strategy where TNFα induces upregulation of the expression of the transmembrane glycoprotein mucin 4 (MUC4) to impair trastuzumab binding, preventing antibody mediated killing of BC cells. Etanercept (E), an inhibitor of TNFα, downregulated MUC4 expression and sensitized de novo trastuzumab-resistant BC xenografts to trastuzumab. The aim of this work was to study whether etanercept improved antitumor innate immune response (IIR) mediated by trastuzumab.
We used the de novo trastuzumab-resistant and TNFα-producing cell line JIMT-1 to establish s.c. tumors in female nude mice. Animals were treated with IgG, T, E or T+E (5 mg/kg each) i.p. twice a week. Treatment with T+E significantly reduced tumor growth in 72,4% ($p<0.01$) vs. the control group, IgG. Spleen NK cells from T+E group showed an increase in the degranulation marker CD107a by flow cytometry ($p<0.05$) vs. IgG. Moreover, spleen NK cells from T and T+E groups showed an enhanced trastuzumab-dependent degranulation in an ex vivo assay ($p<0.01$) vs. IgG. In addition, T+E treatment also reduced total myeloid cells (CD11b+) infiltration in tumor microenvironment (TME) ($p<0.01$) vs IgG, but granulocytic and monocytic subtypes distribution remained unchanged. MUC4 and cyclinD1 expression determined by Western blot were downregulated in tumors treated with T+E ($p<0.01$ and $p<0.05$, respectively) and AKT phosphorylation was inhibited ($p<0.01$) with respect to IgG, T and E.
These results suggest that TNFα blockade downregulates MUC4 expression reduces tumor burden and improves the IIR, increasing NK degranulation and generating a less suppressive TME. Patients with HER2+ MUC4+ BC could be eligible for the combined therapy T+E to overcome/avoid resistance.

485. (616) **TUMOR-SUPPRESSIVE FUNCTIONS OF 4-METHYLUMBELLIFERONE ON HUMAN AML CELLS: MODULATION OF SENESCENCE, CD44 EXPRESSION AND MITOCHONDRIAL STATUS.**
Mariángeles Díaz, Tomas Lombardo, Matías Pibuel, Daniela Poodts, Daniela Laura Papademetrio, Élida Álvarez, Silvia E. Hajos, Silvina Lompardía
Instituto de Estudios de la Inmunidad Humoral - IDEHU (UBA-CONICET)

Beside the improvement in acute myeloid leukemia (AML) therapy, half of patients die due to complications related to treatment. CD44, the main hyaluronan receptor, has been proposed as a therapeutic target in AML. Down-regulation of this receptor has been associated to inhibition of cell proliferation and metabolic reprogramming including mitochondrial changes which sensitize to chemotherapy in different tumors. 4-methylumbelliferone (4MU) has shown promising effects as a potential new drug in cancer. Recently, it has been demonstrated that 4MU down-regulates CD44 expression in breast cancer cells. However, little is known about 4MU effects on hematological malignancies. Previous results of our lab showed that 4MU inhibited cell proliferation in a dose dependent manner in AML cell lines without inducing apoptosis at lower doses but showing senescense-associated heterochromatin foci (SAHF+) cells by DAPI stain. Considering this, we hypothesize that 4MU would be a potential new drug for the treatment of acute leukemia. The aim of this work was to evaluate the effect of low doses of 4MU on CD44 expression, senescence modulation and mitochondrial status in human AML cell line (U937). Results showed that treatment with 4MU reduced significantlyl CD44 expression in a dose dependent manner as assessed by FC ($p<0.05$) and WB. Also, 4MU increased the mean of fluorescence of TMRE ($p<0.05$) and NAO ($p<0.01$) by FC suggesting an increment of mitochondrial mass. Fluorescence microscopy using MitotrackerRed showed mitochondrial elongation, accumulation of these organelles and changes on their distribution. In order to evaluate senescence we performed a SA-β-galactosidase colorimetric assay. 4MU increased SA-β-Gal+ cells percentage ($p<0.05$) in U937 cell lines. In view of these results, we conclude that 4MU down-regulates CD44, with an increment in mitochondrial mass and the induction of senescence in U937 cell line. These findings broaden the knowledge of the potential use of 4MU in acute leukemia treatment.

486. (90) **MAGEB2 ENHANCES RDNA TRANSCRIPTION AND GLOBAL PROTEIN SYNTHESIS**
María Fátima Ladelfa[1, 2], Gastón Ezequiel Amato[1], Franco Andrés Pascucci[1, 2], Martín Monte[1]
[1]*Dpto. de Química Biológica, FCEN-UBA* , [2]*IQUIBICEN, CONICET*