6 months. The aim of this work was to identify protein profiles associated with the resistant phenotype. To this end, we compared BT-474 and BT-474R through a LFQ proteomic approach. A *label-free mass spectrometric* protein *quantification followed by* Proteome Discoverer analyses was performed (FDR 1%). For differential protein expression analysis, we employed DEqMS in R/Bioconductor. Results were visualized in a Volcano plot (fold change≥2). We identified 176 differentially abundant proteins from a total of 1,381, among which 62 were upregulated and 114 downregulated in BT-474R cells ($p<0.05$). Then, to identify the cellular processes involved in this modulation, we performed a GeneSet Enrichment Analysis (GSEA) using clusterProfiler in R/Bioconductor. The most highlighted processes that could be associated with Tz resistance were oxidative phosphorylation, fatty acid metabolism, mTORC1 signaling ($p<0.001$) and G2-M checkpoint ($p<0.01$). Cell cycle proteins were analyzed, finding Cdk1 upregulated in BT-474R cells ($p<0.001$). Furthermore, pirin and DCLK1 proteins, known as positive regulators of cell proliferation and tumor progression, were also upregulated in the resistant cell line. Accordingly, we found that BT-474R cells have a significantly shorter doubling time than BT-474 cell line (30.23 h vs 92.37 h, $p<0.05$). Our findings suggest a more proliferative phenotype associated with Tz-resistant cells. Further studies are needed to understand the proteomic changes involved in resistance acquisition.

BIOINFORMATICS AND THERAPEUTIC TARGETS II

Friday, November 18, 14-15:30 hr

Chairs: María Sol Ruiz - Ayelén Toro - Juan Bizzotto - Fernanda Rubio

32. (6) **THE TRADEOFFS BETWEEN CLINICAL AND LABORATORY BIOMEDICAL RESEARCH**

Oscar Bottasso[1]

[1]*Instituto de Inmunología Clínica y Experimental de Rosario (IDICER, UNR-CONICET)*

The need to achieve a greater understanding of the diverse array of medical problems imposes a kind of conducting force capable of pushing this continuously shifting exploration border towards areas of greater confidence. The science and the medical practice digging their backgrounds through hypotheses, experimental verifications, and subsequent interpretations are presently getting the input of Translational Medicine viewed as a goal of biomedical research aimed at offering better control tools. Something that is closely linked to the origins of the very medicine. The transition between basic and clinical research is complex requiring appropriate crosstalk helpful enough for the input/output relationship to become fruitful. Partly because the *in vitro* experiments and preclinical studies do not necessarily mirror the clinical situation to sensibly translate the "truth" emerging from laboratory data. This is particularly relevant when trying to develop new biomarkers, potentially predictive scoring systems, biostatistical procedures for combined endpoints, and mostly, the efforts towards the availability of novel therapeutic approaches. Regardless of the precise situation, intermingled communication between players from different fields is necessary, if we are about to establish new conceptual and methodological frameworks likely to facilitate a better theoretical/practical approach. To some extent, it constitutes a sort of burst of the biomedical sciences into the clinical scenario aimed at validating the knowledge arising in the laboratory to become part of some clinical practice or guidelines for health policies, with all the implications therein. Conversely, many clinical observations raise questions that can be elucidated from the experimental ground, as a valuable way of affording some feedback on this interactive process. The way ahead appears as long as challenging, given that the bulk of the investigative work carried out in our setting has been mostly performed in self-confined compartments (biomedical, clinical, and public health). Purportedly, we have lastly realized that field-specific speeches concentrated on themselves end up hindering the portrayal of problems in their different facets, as well as the way of formulating investigative designs addressed to provide improved alternatives.

33. (44) **INFLUENCE OF MEMBRANE LIPID COMPOSITION ON WATER DIFFUSION THROUGH HUMAN AQUAPORIN-1**

Casal J.J., Dorr R.A., Toriano R.

Universidad de Buenos Aires. CONICET. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay). Facultad de Medicina, Paraguay 2155 7º (1121) Buenos Aires, Argentina

Introduction. Lipids play important modulatory and structural roles for membrane proteins. Molecular dynamics simulations (MDS) are frequently used to provide insights into the nature of these protein-lipid interactions. We study the influence of the lipid environment on human Aquaporin-1 (hAQP1, PDB code: 4CSK). Two heterogeneous lipid bilayers, representative of mammals (M) and cancer cells (C), and other only with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), were generated around 4CSK. Methods. CHARMM-GUI was used for the generation of the three systems. 4CSK was embedded into a 70 × 70 Å lipid bilayer, with TIP3P water molecule model, neutralizing ions, and a temperature set at 37ºC. The CHARMM36m force field was used at production dynamics of 500 ns. GROMACS, VMD and MDAnalysis were used to evaluate the convergence of the simulation, pore radius profile and water diffusion through the protein channels, distances between selectivity residues (ar/R) and the number of water molecules near them and pore length in the narrow zone. Comparative mean, SD and ANOVA tests were used in statistics. Results. Constriction in the ar/R site: varied from a narrow to a wide conformation. A closed state coincides with the absence of water molecules at the site. Water diffusion through protein in DPPC: 8.3 (0.04), C: 8.62 (0.08) > M: 4.44 (0.01), units: 10^{-14} cm^3s^{-1}. Pore length in C: 20.40 (0.48) > DPPC: 18.94 (0.42) > M: 18.49 (0.37), units: Å. Conclusion. We present quantitative evidence that membrane composition affects AQP1 water dynamics. Our findings confirm the need for further progress in the study of the regulation of aquaporins by their lipid environment. With the emergence of more powerful hardware and advanced simulation techniques and algorithms, we can expect an even larger impact of simulations on our understanding of biological membranes and the role of lipids.

34. (170) **DRUG REPURPOSING STRATEGY TARGETING SEROTONIN-GATED ION CHANNELS**

Noelia Rodriguez Araujo, Guillermina Hernando and Cecilia Bouzat

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)-CONICET, 8000 Bahía Blanca, Argentina.

Drug repurposing is an effective strategy for identifying new therapeutic use(s) for currently available drugs. We focused on serotonin (5-HT)-gated ion channels of nematodes and vertebrates and tested a series of clinically used drugs by electrophysiological techniques. We studied a nematode serotonin-activated chloride channel, MOD-1, as a novel target for antiparasitic drugs, and the vertebrate 5-HT_3A receptor as a drug target for the treatment of nausea, emesis, and irritable bowel syndrome. Receptors were expressed in mammalian cells and their function was measured by whole-cell recordings. Drug screening assays revealed that piperazine (PZE), an anthelmintic drug acting at nematode GABA receptors, decreased macroscopic currents elicited by 5-HT of MOD-1 (IC_{50} 113±29 μM). The analysis indicated that PZE acts as a negative allosteric modulator of MOD-1. Moreover, motility assays using the nematode model *Caenorhabditis elegans* showed that the negative modulation impacts on worm behavior, thus confirming the inhibition of MOD-1 as a novel anthelmintic mechanism. We tested PZE derivatives acting as H1-antihistamine drugs and found that hydroxyzine inhibited MOD-1 responses whereas cetirizine did not have any effect. We also showed that tryptamine, which has significantly higher agonist efficacy for MOD-1 (α=80%) than for 5-HT_3A (α=27%), affected worm motility, indicating that it can be a novel anthelmintic lead compound. Moreover, we found that sumatriptan, a tryptamine-derivative currently used for migraine, also inhibited MOD-1 currents elicited by 5-HT. In 5-HT_3A studies, we found that PZE also decreased macroscopic responses elicited by 5-HT (IC_{50} 238±89 μM), thus revealing a novel

allosteric inhibitor of this receptor. Our drug repurposing approach contributes to identify new targets and novel pharmacological uses of clinical drugs on a rational basis.

35. (279) **IMPACT OF FECAL MICROBIOTA TRANSPLANTATION IN PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS**

María Florencia Fernández[1], Martín Ruhle[1], Daniela Borgnia[1], Claudia Hernandez[2], Ana Juliá[3], Adriana Bottero[4], Laura Busquet[4], Carlos Waldbaum[5], Fabiana López Mingorance[5], Carlos Figueroa Turienzo[3], Andrea Mangano[1]

[1]Unidad de Virología y Epidemiología Molecular-CONICET, Hospital de Pediatría "J.P. Garrahan", Argentina, [2]Servicio de Microbiología, Hospital de Pediatría "J.P. Garrahan", Argentina, [3]Servicio de Trasplante de médula ósea, Hospital de pediatría "J.P. Garrahan", Argentina, [4]Servicio de Gastroenterología, Hospital de Pediatría "J. P. Garrahan", Argentina, [5]Departamento de Gastroenterología, Hospital de Clínicas José de San Martín, Argentina.

Background and Aims: The Graft-Versus-Host-Disease (GVHD) is a major cause of mortality related to Hematopoietic Stem Cell Transplant (HSCT). The conditioning regimen for HSCT leads to disruption of microbiota homeostasis, called dysbiosis. Lower alpha-diversity of the Fecal Microbiota (FM) at the time of neutrophil engraftment was associated with higher transplantation-related mortality. For this reason, numerous therapeutic strategies have been proposed to restore FM integrity, including use of Fecal Microbiota Transplantation (FMT). Our goal is to evaluate the changes FMT produces in FM of pediatric HSCT recipients and describe their clinical evolution. Methods: We monitored 15 pediatric HSCT recipients 7 days before and after FMT, including the donors. Library preparation was based on 16S Metagenomic protocol and Illumina MiSeq sequencing. Metataxonomic and statistical analysis was performed by SHAMAN software. Results: Microbiome alpha diversity, related to detect Operational Taxonomic Units (OTUs), was compared between the 3 groups (donors, pre-FMT and post-FMT). We found α-diversity increased significantly post-FMT (p=0.00045). Donors conferred 48 new OTUs after FMT. Moreover, Bacteroides acquired bacterial dominance (>37.8%) and Ruminococcus increased significantly (1.8% to 17.1%) after FMT. In our cohort, 8 patients developed grade 2 GVHD, 1 patient grade 3 GVHD and 6 did not present GVHD complications. Furthermore, no transplant-related mortality was observed. Conclusions: To our knowledge, this is the first study using metagenomic sequencing in a pediatric cohort undergoing FMT after-HSCT. These findings suggest that increase in diversity results from transferring new OTUs from the donor during the FMT. Despite the fact that there were no HSCT-related deaths or severe GVHD, the small patient population makes it infeasible to correlate to any specific genera. Further FM characterization may provide insights into other factors influencing clinical outcomes.

36. (289) **TRANSCRIPTIONAL ANALYSIS SHEDS LIGHT ON RUSSIAN STURGEON MECHANISMS TO COPE WITH BACTERIAL INFECTION AND CHRONIC HEAT STRESS**

Valeria Silva-Álvarez[1,2], Alicia Costábile[3], Mauricio Castellano[3], Marcio Aversa-Marnai[1,2], Ignacio Quartiani[4], Daniel Conijeski[5], Alejandro Perretta[4], Andrea Villarino[3], Ana María Ferreira[1,2]

[1] Unidad de Inmunología, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República, Uruguay.
[2] Área Inmunología, Departamento de Biociencias, Facultad de Química, Universidad de la República, Uruguay.
[3] Sección Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de la República, Uruguay.
[4] Unidad de Patología, Biología y Cultivo de Organismos Acuáticos, Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de la República, Uruguay.
[5] Esturiones del Río Negro S.A., Uruguay.

Sturgeons are chondrostean fish of high economic value and critically endangered due to anthropogenic activities, which has led to sturgeon aquaculture development. Russian sturgeon (*Acipenser gueldenstaedtii*), the second most important species reared for caviar, is successfully farmed in subtropical countries, including Uruguay. However, during the Uruguayan summer, sturgeons face intolerable warmer temperatures that weaken their defences and favour infections by opportunistic pathogens, increasing fish mortality and farm economic losses. Since innate immunity is paramount in fish, for which the liver plays a key role, we used deep RNA sequencing to analyse differentially expressed genes in the liver of Russian sturgeons exposed to chronic heat stress and challenged with *Aeromonas hydrophila*. We assembled 149.615 unigenes in the Russian sturgeon liver transcriptome and found that metabolism and immune defence pathways are among the top five biological processes taking place in the liver. Chronic heat stress provoked profound effects on liver biological functions, up-regulating genes related to protein folding, heat shock response and lipid and protein metabolism to meet energy demands for coping with heat stress. Besides, long term exposure to heat stress led to cell damage triggering liver inflammation and diminishing liver ability to mount an innate response to *A. hydrophila* challenge. Accordingly, the reprogramming of liver metabolism over an extended period had detrimental effects on fish health, resulting in weight loss and mortality, with the latter increasing after *A. hydrophila* challenge. This transcriptomic study describes how chronic heat stressed sturgeons respond to a bacterial challenge, suggesting that liver metabolism alterations have a negative impact on the innate anti-bacterial response of this ancient fish.

37. (328) **INTEGRATION OF COMPUTATIONAL AND EXPERIMENTAL APPROACHES TO DISCOVER NEW POTENTIAL *TRYPANOSOMA CRUZI* OLIGOPEPTIDASE B INHIBITORS**

Melina Mottin[1,2]; Matheus Silva[1]; Milene Andrade[1]; Carolina Horta Andrade[2]; Jaime M Santana[1], Izabela M. D. Bastos[1]; Flávia S. N. Motta[1,3].

1. Laboratório de Interação Patógeno-Hospedeiro (LIPH), University of Brasília, Brasília, DF, Brazil.
2. Laboratory of Molecular Modeling and Drug Design (LabMol), Faculdade de Farmácia, Universidade Federal de Goiás, Goiânia, GO, Brazil.
3. Faculdade de Ceilândia, University of Brasília, Brasília, DF, Brazil
Corresponding author e-mail: fnmotta@unb.br

Trypanosoma cruzi oligopeptidase B (OPBTc) is proven to participate in the parasite infection process and has been increasingly cited as an important virulence factor in trypanosomatids, since it has no orthologs in mammals. Therefore, OPBTc is a promising target for the rational design of effective and safe inhibitors, with a greater possibility of therapeutic success. In this study, the ChemBridge commercial database was virtual screened (VS) against OPBTc, aiming to prioritize new potential inhibitors. The VS was performed based on the following computational filters: (*i*) structural similarity to known OPBTc inhibitors; (*ii*) molecular docking at the OPBTc catalytic binding site; and (*iii*) lipophilicity (logP) and solubility (logS) properties. Firstly, a similarity search of ChemBridge dataset was performed based on the structure of 13 OPBTc inhibitors, previously identified in our laboratory. A total of 136 compounds were selected for the molecular docking VS. The 3D structure of OPBTc is not experimentally available. Thus, we predicted OPBTc 3D models using different methods: homology modelling, protein folding and *ab initio* (deep learning). Four models were obtained using the Swiss-Model, Itasser, trRosetta and Alphafold servers. All models were evaluated regarding statistical quality parameters, using MolProbity server. The best model was obtained through the Alphafold server. The OPBTc catalytic binding site is composed by the triad His682, Ser562 and Asp647. Then, docking calculations were performed against the best OPBTc 3D model, at the catalytic site, using the virtual screening workflow protocol of the Glide program. The top 10 ranked virtual hits presented docking scores ranging from -8.0 to -2.0 Kcal·mol^{-1}. Lipophilicity and solubility properties of the hits were evaluated and a final hit list with 22 promising OPBTc inhibitors was obtained. Future enzymatic and parasite assays will be performed to evaluate the activity of the prioritized hits against OPBTc.