negative correlation with mIDH1 in the whole set of mIDH gliomas ($p<0.05$). However, within this group, this correlation is completely lost in AA (Spearman r: 0.06) in comparison with OD (Spearman r: -0.46, $p<0.05$). The analysis of gene signatures of tumor-infiltrating immune cells indicated that lymphocyte populations and antigen presenting cells were downregulated in mIDH tumors ($p<0.05$, vs. wtIDH). However, once again, we found differences within the mIDH group, suggesting that OD exhibit an even colder immunophenotype than AA. Finally, we analyzed the correlation between the expression of IDH1 and MGMT or ATM, DNA repair enzymes that affect chemo- and radio-resistance, respectively. We found a significantly negative correlation between IDH1 and MGMT that is lost in GBM biopsies. In contrast, IDH1 exhibited a significantly positive correlation with ATM in mIDH gliomas but a negative one in GBM. Our observations suggest that the immune landscape of gliomas not only differs due to mIDH status, but also within glioma subtypes, supporting the idea that the overall effect of this genetic lesion depends on the cellular context.

439. (069) **cAMP EFFLUX INHIBITION BY NSAIDS: DRUG REPOSITIONING FOR PDAC TREATMENT**

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In a previous work, we validated the inhibition of MRP4-dependant cAMP extrusion process as a promising therapeutic strategy for Pancreatic Ductal Adenocarcinoma (PDAC). In view of the therapeutic challenge associated with PDAC, we set out to search and characterize approved drugs that inhibit cAMP transport with the goal of establishing a repositioning strategy. Based on the results of this screening, we selected the Non-steroidal anti-inflammatory drugs (NSAIDs) as an interesting pharmacological family to inquire for rational drug repositioning. NSAIDs have been tested in the past as co-adjuvants in the therapy of various types of cancer, in many cases with positive results. Although their effects that depend on cyclooxygenase-2 inhibition are well described, the effects that are independent of this inhibition are far for being clear. We hypothesize that MRP4 inhibition could be a missing link in the overall action on tumor progression of these compounds. In this work, we measure the intracellular cAMP response upon treatment with 13 different NSAIDs using a technique developed in our laboratory in which we use HEK-293T cells stably transfected with the EPAC-SH187 sensor. Ibuprofen, acetyilsalicylic acid, Naproxen, Indomethacin, Diclofenac, Dexketoprofen and Ketorolac have shown to increase intracellular cAMP concentrations upon treatment ($p<0.01$). The concomitant significant reduction of extracellular cAMP upon treatment with these NSAIDs was also measured using a Radio-Binding Protein assay (RBP), which confirmed cAMP transport inhibition as one of the mechanisms that triggers intracellular cAMP increment ($p<0.05$). On the other hand, Celecoxib, Acetaminophen, Dipyrone, Phenacetin, Meloxicam and Piroxicam failed to increase intracellular cAMP upon treatment. These emerging results, together with an exhaustive literature search will allow us to select our repositioning candidates to continue with its characterization regarding its therapeutic potential in PDAC.

440. (070) **iNOS INHIBITOR S-METHYLISOTHIOUREA AFFECTS GLIOBLASTOMA STEM CELL NICHE, WITHOUT AFFECTING DIFFERENTIATED TUMOR CELLS**

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Introduction: Glioblastomas (GBM) are the most common and aggressive brain tumors. Despite the traditional chemotherapy with temozolomide (TMZ) and radiotherapy, patients' survival does not exceed 2 years. It has been suggested that the selective inhibition of inducible nitric oxide synthase isoform (iNOS) enzyme is related to a decreased proliferation of GBM cells.

Objective: Evaluate the effect of iNOS specific inhibitor, S-methylisothiourea (SMT), alone or in combination with TMZ, on GBM stem cell (GSC) niche (spheres) and on more differentiated cells (monolayer and spheroids).

Methodology: Human GBM cell lines LN229, U251 and U87 were seeded in monolayer, as spheroides (hanging drop aggregation assay) and under sphere conditions (low adhesion and high dilution). Viability in 2D was determined by MTS. For spheroids' growth monitoring, they were measured in their diameter every week using imageJ. The number of GSC was established by sphere forming efficiency (SFE) in relation to the seeded cells, and the diameter of the spheres was also measured.

Results: In monolayer and in spheroids, SMT (50 μM) marginally reduced LN229 cell line growth (20-5% inhibition); however, in GSC niche, it decreased significantly the SFE in the three lines (inhibition, LN229 42%; U251 61%; U87 48%) and their diameters (inhibition, 33%, 17% and 28%, respectively). The combination of SMT (50 μM) with TMZ (250 μM), further inhibited SFE (LN229 57%, U251 70%, U87 50%) compared to SMT or TMZ alone.

Conclusion: iNOS inhibitor in combination with TMZ therapy, could be useful in reducing GSC growth . Further studies on mechanisms of action will establish the differences observed between different GBM cell lines.

441. (079) **BRAF AND HISTONE 3 ALTERATIONS IN THE INTEGRATED DIAGNOSIS OF PEDIATRIC GLIAL AND GLIONEURONAL TUMORS: A SINGLE CENTER EXPERIENCE**

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While tumors of the central nervous system account for over 20% of pediatric tumors, gliomas represent more than 55% of them. Their classification into low (LGG) or high grade gliomas (HGG) may reflect survival odds. Molecular techniques enable more accurate diagnostic results and risk stratification. Molecular alterations in *BRAF* gene and histone 3 genes (H3) were evaluated by FISH, IHC and Sanger sequencing, in 102 pediatric glial and glioneuronal tumors. BRAF and/or H3 were assessed in LGG or HGG according to WHO recommendations. Results were correlated with clinical and histological findings to evaluate them as diagnostic and prognostic tools. The *KIAA1549-BRAF* gene fusion was relevant as a diagnostic tool for Pilocytic astrocytoma, a LGG,(43/64 cases), but was not related to progression free survival (PFS) and overall survival (OS) (Kaplan Meier, Log-rank
(Mantel-Cox) Test; P>0.05). This fusion showed no association with different age groups (10
< years old ≥ 10, Fisher's exact test; P>0.05), but was more prevalent in the cerebellum (Chi square test; P=0.04). The BRAFV600E mutation occurred preferentially in the brain hemispheres 7/10 cases (Fisher´s exact test; P=0.004) and was associated with a shorter OS (P=0.0082), but not with a decreased PFS (P=0.14) in LGG. When only considering Pilocytic astrocytomas, it was associated with a decreased OS and PFS (P<0.0001 and P=0.0135, respectively). All HGG of the midline were positive for H3K27M mutation, while the H3G34R mutant cases were located in brain hemispheres. The H3K27M mutation in HGG was associated with decreased PFS and OS (P=0.0124 and P=0.006, respectively).
Assessing druggable molecular markers with prognostic value is of paramount importance particularly in those cases where complete resection or further radiation therapy is not possible due to critical-location of the tumor.