for short.
Cytotoxic activity on MG-63 cell line was evaluated in 2D (monolayer) and 3D (spheroids) models. Cu(HL) significantly reduced cell viability after 24 h treatment in both models (IC_{50} 2D: 1.98 ± 0.51 μM; 3D: 9.05 ± 1.0 μM) ($p<0.001$). Further studies demonstrated that Cu(HL) inhibits cell proliferation and conveys cells to apoptosis, determined by flow cytometry. Cu(HL) showed a great genotoxicity, evaluated by comet assay.
Finally, we assessed *in vivo* anticancer activity in animals bearing growing OS s.c. xenografts. Treatment during 4 weeks with Cu(HL) (2 mg/kg i.p. three times per week) markedly impaired tumor progression, enhancing necrosis and reducing tumor growth rate and mitotic index ($p<0.01$). Treatment with an equivalent low dose of reference metallodrug cisplatin (2 mg/kg i.p. three times per week) failed to inhibit tumor growth.
Taken together, these results show that Cu(HL) has a promising anticancer activity against *in vitro* and *in vivo* OS models.

439. (058) **IMPACT OF IDH MUTATIONS ON THE IMMUNOLOGICAL LANDSCAPE OF GLIOMAS: A TCGA META-ANALYSIS BASED ON THE 2021 WHO CLASSIFICATION OF BRAIN TUMOURS**

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Mutations in the enzyme isocitrate dehydrogenase genes (mIDH) are currently used to classify diffuse gliomas, the most common malignant primary brain tumours in adults. Additional genetic lesions led to the most recent WHO classification that allows stratification in four tumor entities, mIDH gliomas [Oligodendrogliomas (OD) and Astrocytomas (AA)], and wtIDH gliomas: [Glioblastoma (GBM)-like and GBM]. While mIDH clearly correlates with better prognosis, the role of this mutation in antitumor immunity remains controversial. First, we predicted the level of infiltrating immune and stroma cells by ESTIMATE scores and both were significantly lower in mIDH patients. We also found reduced expression of immunoregulatory genes (PD-LI, PD-1, CTLA-4, IDO1, IL-10, LAG3 and TIM3) in mIDH biopsies ($p<0.05$, vs. wtIDH). Moreover, PD-L1 exhibited a strong 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.

440. (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.

441. (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.