Introduction A panoply of studies have been indicating that estrogens are protective agents in prostate carcinogenesis. However, the physiological effects of estrogens in PCa mainly have been associated with the differential activation of the nuclear estrogens receptors (ER), with much less knowledge existing on the membrane ER. The G protein-coupled ER (GPER), known to be involved in the rapid nongenomic responses, has been linked to antiproliferative and proapoptotic effects and is a likely candidate mediating the ‘anti-carcinogenic’ actions of estrogens. This work aims to characterise the GPER role controlling proliferation, apoptosis and metabolism of PCa cells.

Material and methods The nonneoplastic PNT1A cell line and neoplastic LNCaP DU145 and PC3 cell lines were maintained in culture in RPMI 1640 medium. GPER expression pattern was characterised by Western blot. Fluorescent immunocytochemistry allowed determining the subcellular localization of GPER by colocalization with wheat germ agglutinin, calnexin, and hoescht. PNT1A, LNCaP, DU145 and PC3 cells were treated with the GPER specific agonist G1 (1 μM) for 24 hour. Cell viability was assessed by the MTT assay. The effect of GPER activation on cell proliferation, apoptosis and metabolism was assessed by analysing the expression of key proteins in each process. Also, the enzymatic activity of caspase-3 and LHD was measured. Glucose consumption and lactate production were determined using commercial kits.

Results and discussions GPER was differentially expressed in PCa cell line models depending on their aggressiveness and disease status. GPER expression was highest in the androgen-sensitive and less aggressive LNCaP cells and decreased in the more aggressive castration-resistant cell line models (DU145 and PC3). GPER was located at the cell membrane, endoplasmic reticulum, and also in the nucleus. The activation of GPER by G1 decreased PCa cells viability, concomitantly with altered expression of key regulators of proliferation and apoptosis. Furthermore, G1-stimulated cells displayed augmented caspase-3 activity comparatively to the control group. G1 treatment also modulated PCa cell metabolism with altered glucose consumption and lactate production.

Conclusion GPER activation decreased viability of PCa cells whereas enhancing apoptosis. Also, PCa metabolic profile was altered in response to G1. These findings stimulate further research to ascertain the role of GPER as a therapeutic target.

Abstract Withdrawn
which correlates strongly with poor prognosis across a wide variety of cancers. We postulated that deletion of MPC may force ovarian cancer cells to use glutamine as a fuel source enabling their survival in glucose limited environments.

**Material and methods** Using ovarian cancer cell lines characterised as glutamine-addicted (SKOV3) or glutamine-independent (OVCA3) as exemplars, the impact of MPC1 on ovarian cancer cell metabolism was investigated by quantitative PCR, Western blotting, metabolic assays and the Seahorse XF Analyzer.

**Results and discussions** The importance of glutamine to an invasive phenotype is indicated by the observation that SKOV3 cells but not OVCAR3 cells were migratory in the presence of glutamine. The functional significance of MPC as an important link between glycolysis and OXPHOS was shown by inhibiting MPC biochemically with UK5099. UK5099 altered glutamine-independent OVCAR3 cells to emulate SKOV3 cells driving a switch to glutamine metabolism for OXPHOS. Whereas, non-inhibited OVCAR3 cells, even in glucose free media, did not utilise glutamine for OXPHOS.

**Conclusion** The ability to model the switch from glutamine-independent to glutamine-addicted in ovarian cancer cells will allow us to investigate the metabolic and genetic changes that occur in progression from a low- to a highly-invasive cancer phenotype of ovarian cancer. This in turn will provide therapeutic targets to halt or slow ovarian tumour progression.

**PO-258**

**TELMISARTAN INDUCES MELANOMA CELL APOPTOSIS VIA GENERATION OF ROS AND HAS SYNERGISTIC EFFECTS WITH TARGETED THERAPY IN VITRO**

J Grahovac*, T Srdić-Rajić, M Pavlović, S Radulović. Institute for Oncology and Radiology of Serbia, Experimental Oncology, Belgrade, Serbia

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**Introduction** Melanoma is one of the most aggressive malignancies. A half of melanomas carry BRAF V600E mutation, but despite dramatic initial effects of BRAF inhibitors in the clinic, patients eventually relapse, suggesting that combination therapies are needed to overcome resistance. BRAF inhibitors suppress glycolysis, yet the subsequent increase in oxidative metabolism limits their efficacy. Therefore, agents that target different aspects of cell metabolism may improve melanoma therapy efficacy. Telmisartan is the AT1R inhibitor and PPARγ agonist. PPARγ is a nuclear receptor that is an important regulator of lipid and glucose metabolism. Activation of PPARγ in melanoma cells has growth inhibitory effect, by inducing cell cycle arrest. We hypothesised that telmisartan could inhibit melanoma cell viability and potentially improve BRAF inhibitor therapy by modulating cell metabolism.

**Material and methods** Publicly available databases were analysed for expression of AT1R and PPARγ in melanoma patient tumour samples and various cell lines. A panel of melanoma cell lines was tested in MTT, apoptosis and metabolic assays in presence of telmisartan by flow cytometry.

**Results and discussions** Both telmisartan targets were expressed in melanoma patient samples, but PPARγ levels significantly decreased in melanoma compared to uninvolved skin. Telmisartan decreased viability of melanoma cells in vitro to a similar extent as the direct PPARγ agonist pioglitazone, while pure AT1R inhibitor losartan had no influence. While pioglitazone induced cell cycle arrest in A375 cells, telmisartan induced apoptosis at same concentrations through induction of ROS and dissipation of mitochondrial potential. As expected for PPARγ activation, telmisartan increased glucose uptake in A375 cells. Melanoma cells have intrinsically high levels of oxidative stress due to the accelerated metabolism, which renders them more susceptible to oxidative stress-induced cell death than the normal cells. In line with this notion, telmisartan-induced cell death could be ameliorated with N-acetyl cysteine. In addition, telmisartan synergized with both dacarbazine and vemurafenib in vitro and was still effective in A375R cell line, vemurafenib resistant cell line.

**Conclusion** Telmisartan has anti-melanoma potential in vitro and could increase the effectiveness of melanoma therapeutics, both conventional and BRAF inhibitor therapy. Our future efforts include elucidation of the exact mechanism by which telmisartan exerts these effects.

**PO-259**

**INHIBITION OF THE HEXOSAMINE BIOSYNTHETIC PATHWAY BY TARGETING PGM3 CAUSES BREAST CANCER GROWTH ARREST AND APOPTOSIS**

F Chiardonna*, 1F Ricciardiello, 2G Votta, 2R Palorini, 3R Raccagni, 3H Brunelli, 4L De Gioia, 2R Pastorelli, 5RM Moresco, 5La Ferla. 1University of Milano-Bicocca, Biotechnology and Biosciences, Milano, Italy; 2IRCSS San Raffaele Scientific Institute, Experimental Imaging Center, Milano, Italy; 3Istituto di Ricerca Farmacologiche Mario Negri, Environmental Health Sciences, Milano, Italy; 5University of Milano-Bicocca, Department of Biotechnology and Biosciences, Milano, Italy

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**Introduction** Cancer aberrant N- and O-linked protein glycosylation, frequently resulting from an augmented flux through the Hexosamine Biosynthetic Pathway (HBP), play different roles in tumour progression. Recent studies reported an association between the tumorigenic potential, metastasis and chemoresistance of several type of breast cancer cells and tumours, among which the Triple Negative Breast Cancer (TNBC), and the alteration of their membrane glycans composition and ramification as well as of their level of protein O-GlcNAc. However, the low specificity and toxicity of the existing HBP inhibitors prevented their use for cancer treatment.

**Material and methods** In order to identify a novel inhibitor of HBP pathway and in particular of the PG3 enzyme, we performed a virtual screening by using computational approaches. These approaches lead us to the identification of a lead compound. This compound, named FR054, has been synthetized and in vitro and in vivo tested by using several biophysical methods (NMR, LC/MS, HPLC) and biochemical assay (CETSA, ITDRF, FACS analysis) as well as tested in TNBC xenograft mice model.

**Results and discussions** Here we report the preclinical evaluation of FR054, a novel inhibitor of the HBP enzyme PG3, with a remarkable anti-breast cancer effect. In fact, FR054 induces in different breast cancer cells a dramatic decrease in cell proliferation and survival. In particular, in a model of Triple Negative Breast Cancer (TNBC) cells, MDA-MB-231, we show that these effects are correlated to FR054-dependent reduction of both N- and O-glycosylation level that cause also to a strong reduction of cancer cell adhesion and migration. Moreover we show that impaired survival of cancer cells upon FR054 treatment is associated with activation of the Unfolded Protein Response (UPR) and accumulation of intracellular ROS. Finally, we show that FR054 suppresses cancer growth in MDA-MB-231 xenograft mice.