evaluating these and other miRNAs in patients that follow a second line therapy with palbociclib for at least 2 years or till a new relapse. In summary, we postulate that high levels of S6 and Rb could anticipate which patients will respond to selective kinase inhibitors and could constitute relevant predictive markers for clinical application. Moreover, circulating miRNAs that are associated with S6 and CDK4-6 are good candidates to follow patients under therapy to anticipate tumor relapse.

545. (525) **THE BLOCKAGE OF THE IL6-STAT3 PATHWAY RESTORES TRASTUZUMAB RESPONSE OF HER2+ RESISTANT TUMORS**

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Introduction: Resistance to the Mob Trastuzumab (TZM) is the main cause of dead in HER2+ breast cancer patients and it occurs in around 30% of primary and up to 70% of metastatic tumors. Several pathways are involved in this resistance, and new drugs are used as second and thirst line of treatment. Previous in vitro studies have suggested that the IL6-STAT3 pathway could be involved in TZM resistance, probably by altering the stem cell proportion in the tumor. In the present work we demonstrate in a HER2+ PDX model generated in our lab, that the inhibition of IL6 receptor (IL6R) restores the TZM response in resistant tumors. M&M: A human HER2+ breast tumor was implanted in an immunosuppressed mice, and then transplanted to generate a HER2+ PDX line. ER, PR and KI67 status was confirmed by IHC. PDX mice were afterwards treated with increasing doses of TZM to obtain a resistant tumor. Mob Tocilizumab was used to inhibit IL6R. Results: The TZM resistant tumors were able to grow and resist almost doubled doses of TZM (*p=0,045*). To evaluate the effect of the blockage of the IL6-STAT3 pathway, we applied Tocilizumab (Tocili) that is used in clinic to treat advanced Covid19 patients and rheumatoid disease. The use of Tocili alone generated a small reduction in the tumor growth of TZM resistant tumor (*p=0,009*), suggesting a basal effect of the drug. And the combination of Tocili + TZM generated a stronger reduction in the kinetic of growth when compared to TZM alone (*p=0,004*), similar to the non-resistant tumor. Conclusion and perspectives: the blockage of IL6-STAT3 pathway can restore the sensitivity to TZM in a human HER2+ resistant tumor, suggesting the possible repurposition of Tocilizumab as a treatment for TZM resistant patient. Actually, we are expanding the analysis to more HER2+ PDX tumors.

546. (543) **ESTROGEN INDUCES ID4 SILENCING THROUGH PROMOTER METHYLATION IN ER+ BREAST TUMORS**

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Background: Inhibitor of differentiation protein 4 (ID4) is a dominant negative regulator of the basic helix-loop-helix (bHLH) family of transcription factors. Our group has previously shown that, in breast cancer, ID4 behaves as a tumor suppressor only in estrogen receptor positive (ER+) tumors and that ID4 expression is downregulated through methylation. Taking these observations into consideration, we decided to explore into the study of the molecular mechanisms that lead to the silencing of ID4 through methylation. Given that ID4 is methylated only in ER+ tumors, we hypothesize here that estrogen via estrogen receptor α induces ID4 methylation. Methods: In vitro experiments involved cell culture of MCF7 and T47D ER+ breast cancer cell lines, cells were treated with estradiol, 5-Azacitidine or Tamoxifen. Gene and protein expression were analyzed by RT-qPCR and western blot and methylation by ddMSP. In-silico analyses involved the evaluation of ID4 expression and methylation status on human breast tumors of public datasets according to breast cancer molecular classification and estrogen receptor status. Results: Estradiol treatment induced a reduction in ID4 expression through increased methylation on ID4 promoter ($p<0.05$). 5-Azacitidine induced a reduction in ID4 methylation which was reverted by estradiol treatment in line with a methylating role of estrogens ($p<0.05$). To confirm that the effects induced by estradiol were through the estrogen receptor α, cell lines were treated with Tamoxifen (an antagonist of ER in breast). Tamoxifen treatment decreased ID4 methylation and increased its expression ($p<0.01$). Conclusions: Our findings reveal that estrogens through estrogen receptor α induce the silencing of the tumor suppressor gene ID4 through methylation of its promoter in ER+ breast tumors.

547. (546) **E2F1 AND RB ARE COMMON MEDIATORS OF THE INHIBITORY EFFECTS PROMPTED BY THE COMBINATION OF MIFEPRISTONE AND PALBOCICLIB ON BREAST CANCER CELLS EXPRESSING PROGESTERONE RECEPTOR ISOFORM A**

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Palbociclib (PALBO), a CDK 4/6 inhibitor, is currently used in combination with endocrine therapy targeting estrogen receptors to treat advanced luminal breast cancer. However, with time tumors become resistant to these treatments highlighting the need to develop other therapeutic strategies. Our laboratory focuses on the use of therapies targeting progesterone receptors (PR). We have previously shown that PALBO inhibits luminal breast cancer cell proliferation regardless of the prevailing PR isoform expressed and that mifepristone (MFP), an antiprogestin, potentiates this effect only in cells expressing PR isoform A (PRA). The aim of this study was to evaluate the role of two key cell cycle proteins, RB and E2F1 as mediators of this effect. T47D-YA or T47D-YB cells, expressing respectively PRA or PRB were treated with MFP, PALBO, or MFP+PALBO. The expression of E2F1 and pRB was evaluated by western blots. In agreement with data obtained in cell proliferation studies, a significant decrease of both protein levels ($p<0.05$) was observed only in T47D-YA cells treated with MFP+PALBO, whereas slight decreases were noted with single treatments. Contrarily, in T47D-YB cells, the effects of combined drugs were similar to those induced by PALBO. The *in vivo* growth of T47D cells expressing equimolar levels of PRA and PRB was also inhibited by the combined therapies and the strongest inhibition of pRB was registered by immunohistochemistry in tumors treated with both agents. Our results suggest that E2F1 and RB are key players mediating the inhibition of cell proliferation induced by PALBO and MFP combination exclusively in PRA-expressing cells. Mechanistic studies are underway to explore the direct involvement of PRA on the E2F1 promoter.

548. (551) **REGULATION OF HORMONE-RELATED PROTEINS IN TUMOR-ADJACENT BREAST TISSUE BY MIFEPRISTONE TREATMENT**

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Preclinical data suggests that antiprogestins inhibit the growth of luminal breast carcinomas expressing higher levels of progesterone receptor (PR) isoform A (PRA) than isoform B (PRB), named PRA-H. Therefore, we designed a window-of-opportunity trial (MIPRA; NCT02651844) in order to study the benefits of mifepristone