Analysis of Wnt5A/ROR1 Signaling Inhibition as Cancer Stem Cell Targeting Therapy for Metastatic Prostate Cancer Using Live Organoid Time Lapse Microscope Imaging.

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BACKGROUND-- Wnt5A and its non-canonical Wnt receptor, ROR1, have emerged as a promising new signaling pathway target for lethal, metastatic prostate cancer. Wnt5A has emerged as a significant marker of poor prognosis in circulating tumor cells (CTCs) of metastatic castration resistant prostate cancer (mCRPC) patients. ROR1 is expressed in lethal types of mCRPC. A therapeutic ROR1 antibody, Zilovertamab, has been clinically tested in chronic lymphocytic leukemia (CLL) and metastatic breast cancer and shown to be safe. We are investigating Zilovertamab-based therapies for metastatic prostate cancer.
HYPOTHESIS -- Wnt5A/ROR1 signaling may mediate a cancer stem cell program which makes cells resistant to therapies which target the cell cycle and proliferation. Blocking ROR1 may reveal vulnerabilities which sensitize cancer cells to chemotherapies like docetaxel.
METHODS-- ROR1 expression was determined using RNASeq, qRT-PCR, FACS, IHC and Western blotting. ROR1 signaling was blocked using the anti-ROR1 therapeutic antibody, Zilovertamab, or CRISPRCas9 ROR1 knock out. Cell growth was measured using an Incucyte real time imaging system. Number and size of organoids were determined using Keyence microscope imaging. Cell cycle analysis was performed in live cells in 2D cultures and 3D organoids using the ***Fucci2BL*** bicistronic, **F**luorescent, **U**biquitination-based **C**ell **C**ycle **I**ndicator system in time course assays in an Incucyte and confocal time lapse imaging of live 3D organoids in a stage top incubator unit. PDX PCSD13 tumor growth was measured via calipers and IVIS. RNASeq performed on tumors.
RESULTS-- We showed that ROR1 was expressed at high levels in mCRPC cell lines: PC3, DU145, and in the bone metastatic prostate cancer PDX: PCSD13. CRISPR/Cas9 Knock out of ROR1 in PC3 and DU145 cells showed increased sensitivity to docetaxel inhibition of proliferation in vitro in 2D real time Incucyte proliferation assays and in 3D organoids. Organoid size and number were significantly reduced in ROR1 KO cells. **FUCCI** live cell cycle tracker showed docetaxel led to G2 arrest and ROR1 signaling inhibition increased efficacy of docetaxel induced cell cycle arrest. PC3ROR1KO organoids showed dysfunctional cell division in time lapse confocal imaging. PCSD13 PDX mice treated with Zilovertamab plus docetaxel synergistically increased tumor growth inhibition in vivo and modulated cancer stem cell and cell cycle expression profiles.
CONCLUSIONS-- Therapeutic targeting of these tumor initiating stem/progenitor cells may prevent the evolutionary diversification of a tumor and overcome a critical clinical barrier to cancer treatment. We showed synergistic response in our PDX and cell line models with Zilovertamab plus docetaxel. A phase 1b clinical trial with zilovertamab plus docetaxel in patients with metastatic CRPC (CirmD, NCT05156905, PI R Mckay) is in progress.

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