Disulfiram Enhances Palbociclib and Abemaciclib Activity Through MGMT and CDK4/6 Inhibition in Breast Cancer Cells

- Santhi D Konduri1,2,3, Deborah L Donohoe1,2,3, Dmitry Bosenko1,2,3, Max Albiero4, Tarun Jelia5, Olivia Fukui6, Richard A Rovin1,2,3, Amin B Kassam1,2,3 and George C Bobustuc1,2,3

1 Aurora Cancer Care, 2 Aurora Research Institute, 3 Aurora Neuroscience Innovation Institute, 4 University of Wisconsin, Madison, WI, 5 Case Western, Cleveland, OH, 6 Wake Forest, Winston-Salem, NC, 7 Synaptic Medical, Toronto, CA

**PROBLEM**
Endocrine therapy is the main upfront option for hormone receptor positive breast cancer but some of the patients developing hormonal refractory disease. Therefore, it is important to identify ways to restore estrogen receptor function and/or treat hormonal refractory disease.

**BACKGROUND**
MGMT (O6-methylguanine DNA methyltransferase), a DNA repair protein leading to chemotherapy resistance, is increasingly studied for its cell cycle regulatory role functions, also known to control ER expression and function, is overexpressed in a majority of cancers, including breast cancer. MGMT inhibition has been reported to restore ER function and sensitivity to hormonal therapy in tamoxifen resistant breast cancer. CDK4/6 is a cell cycle regulator targeted by a new class of drugs in the treatment of breast cancer in patients who had progressed during prior endocrine therapy. We investigated a potential correlating role between MGMT and CDK4/6 expression/activity. In this therapeutic context MGMT inhibition would have the dual role of increasing/restoring effect of endocrine therapy and facilitate activity of CDK4/6 inhibitors (Palbociclib and Abemaciclib).

**OBJECTIVE**
To investigate better therapeutic option for hormonal sensitive and hormonal refractory breast cancer.

**METHODS**
We have tested the effect of Amtubase (disulfiram, DSF), as an MGMT inhibitor, at nontoxic doses, on the expression of CDK4/6, or in combination with Palbociclib (PB) or Abemaciclib (LV2835219 - LY) on ER+ breast cancer cells.

**RESULTS**
DSF at very low doses (achievable in human serum with standard DSF clinical dosing) decreases ER+ breast cancer cell growth (MCF7, T47D and ZR75) in a dose-dependent manner. DSF further sensitizes breast cancer cells to PB or LY and significantly inhibits breast cancer growth without causing unwanted side effects on the normal breast epithelial cells. Dose effect and isobologram studies confirm synergistic activity of DSF + LY and moderate synergism for DSF + PB. DSF alone or in combination with PB (DSF + PB) and/or LY (DSF + LY), significantly inhibits expression of MGMT, CDK4/6, ERα and aldehyde dehydrogenase activity – all involved in breast cancer cell cycle proliferation and tumorigenesis. Furthermore, PB and LY dose dependently decreased MGMT and CDK4/6 expression in breast cancer cells and significantly accumulated breast cancer cells in G1 phase of the cell cycle. DSF alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) caused significant apoptosis in breast cancer cells. DSF inhibited colony formation which was further enhanced by addition of PB/LY (DSF + PB/LY). Similarly, DSF alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) decreased the metastatic potential of breast cancer cells.

**CONCLUSIONS**
- DSF at very low doses (achievable in human serum with standard DSF clinical dosing) increased ER+ breast cancer cell growth (MCF7, T47D and ZR75) in a dose-dependent manner.
- DSF further sensitizes breast cancer cells to PB or LY and significantly inhibits breast cancer growth without causing unwanted side effects on the normal breast epithelial cells.
- Dose effect and isobologram studies confirm synergistic activity of DSF + LY and moderate synergism for DSF + PB.
- DSF alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) significantly inhibits expression of MGMT, CDK4/6, ERα and aldehyde dehydrogenase activity – all involved in breast cancer cell cycle proliferation and tumorigenesis.
- Furthermore, PB and LY dose dependently decreased MGMT and CDK4/6 expression in breast cancer cells and significantly accumulated breast cancer cells in G1 phase of the cell cycle.
- DSF alone or in combination with PB (DSF + PB) and/or LY (DSF + LY) caused significant apoptosis in breast cancer cells.
- DSF inhibited colony formation which was further enhanced by addition of PB/LY (DSF + PB/LY).

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