Targeted Degradation of EZH2 leads to Reduced Tamoxifen Resistance in ER-Positive Breast Cancer

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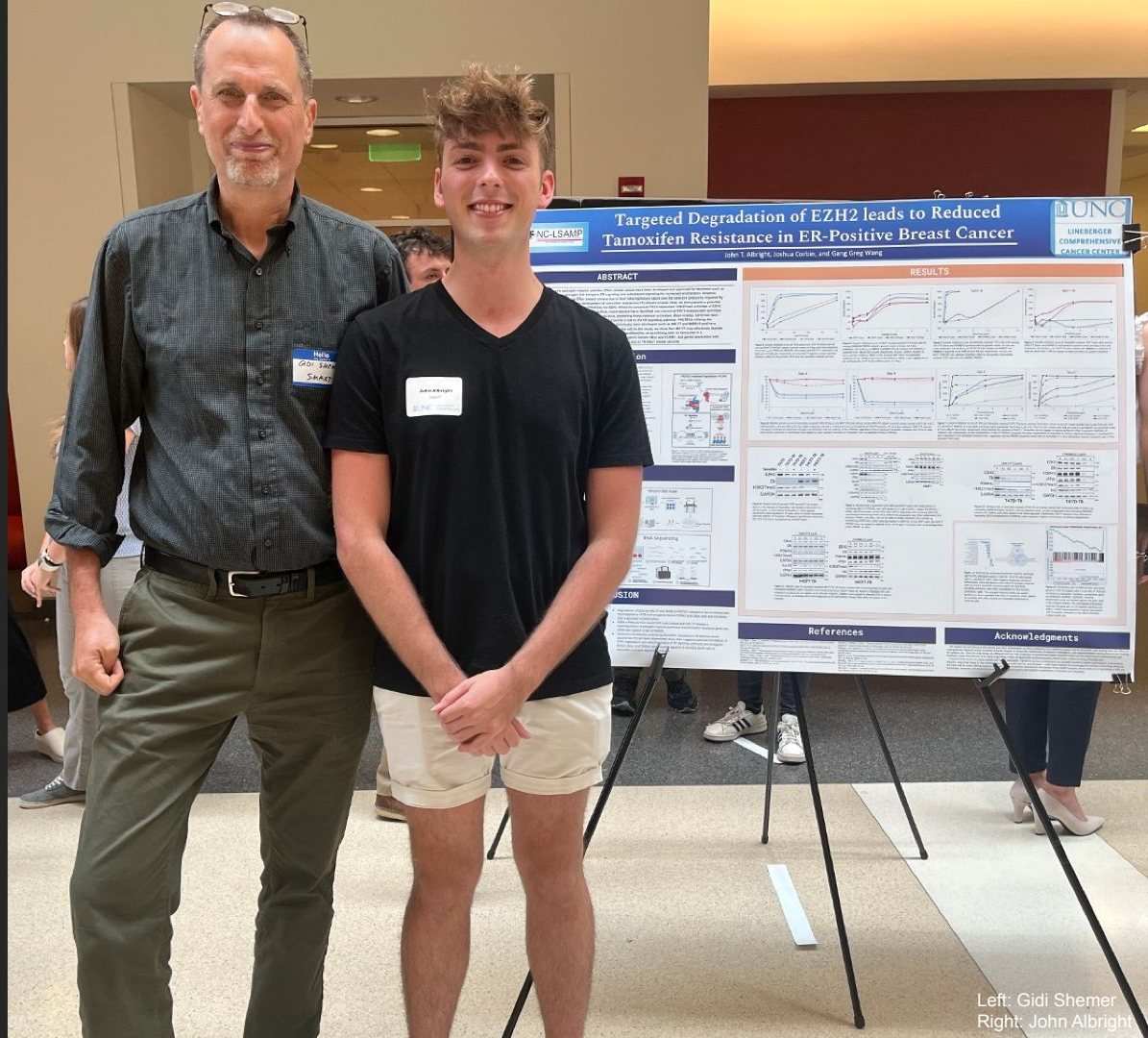
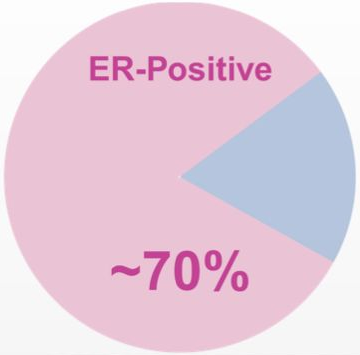
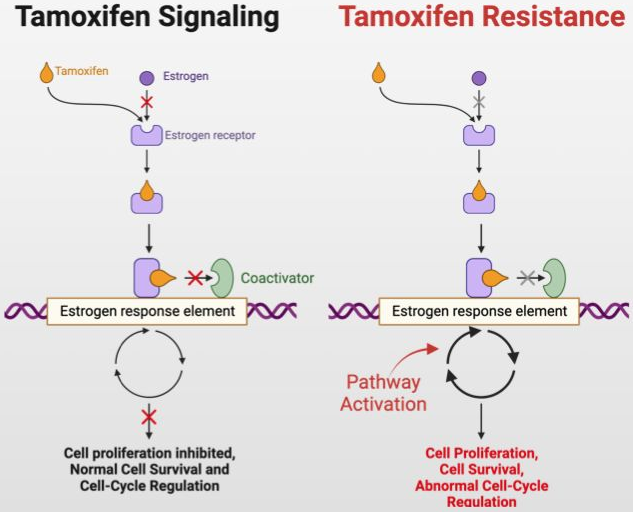


Left: Gidi Shemer
Right: John Albright

Resistance in ER-Positive Breast Cancers

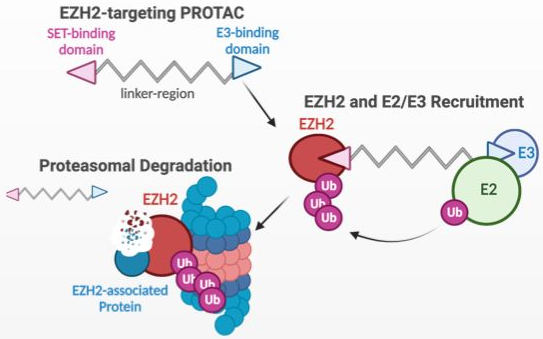


Approximately 70% of all breast carcinoma cases are Estrogen Receptor Positive, and are commonly treated with aromatase inhibitors or selective estrogen receptor modifiers such as Tamoxifen.

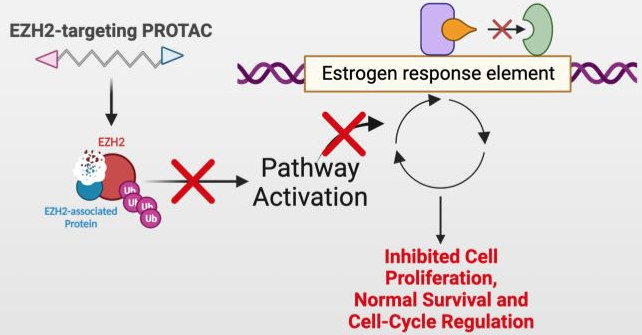


While endocrine agents like Tamoxifen have shown to be effective in treating ER-positive breast cancer, 20-30% of cases develop Resistance to Tamoxifen.

EZH2 PROTAC as a Solution



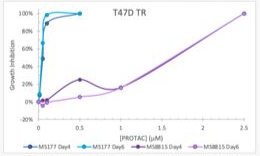
Proteolysis Targeting Chimeras (PROTACs) are small molecules that can bind a protein of interest (POI) and facilitate its degradation. Recent studies have found that PROTACs may also facilitate the degradation of factors that associate with the POI as well.



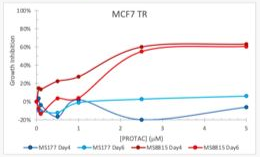
Because EZH2 is known to interact with ER-signaling and associate with oncogenic transcription factors, a PROTAC targeting EZH2 could facilitate downregulation of estrogen responsive genes in ER+ breast cancers, and could interfere with pathways conferring Tamoxifen resistance.

Visualization of Growth Inhibition in Estrogen Receptor Positive Breast Cancer Cells treated with MS177 or MS8815 PROTAC, and Dependance on Tamoxifen.

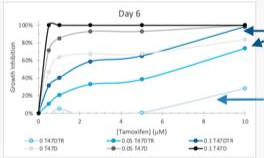
Below are plots showing the growth inhibition (GI) of Tamoxifen resistant ER+ breast cancer cells T47D and MCF7. The first two plots display the dependance of GI on the concentration of either MS177 or MS8815 PROTACs, while the last plot displays GI dependance on concentration of Tamoxifen for T47D cells, for varying treatment with MS177 PROTAC.



The MS177 PROTAC (blue) is more effective at inhibiting growth of the T47D-TR cells than the MS8815 PROTAC (purple). Both PROTACs inhibit growth in a dose dependant manner.



The MS8815 PROTAC (red) inhibits growth of the MCF7-TR cells in a dose-dependant manner, but the MS177 PROTAC does not appear to inhibit growth. Moreover, growth inhibition by MS8815 is less significant than the inhibition seen by MS177 in the T47D-TR cells.



The MS177 PROTAC sensitizes the T47D-TR cells to Tamoxifen in a dose-dependant manner.

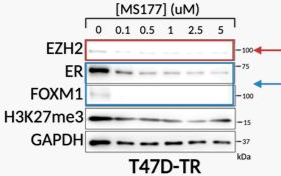
Tamoxifen does not appear to significantly inhibit growth of the T47D-TR cells.

Visualization of Protein Expression in Estrogen Receptor Positive (ER+) breast cancers treated with MS177 or MS8815 PROTAC

Below are immunoblot assays that display protein expression for the Tamoxifen resistant ER+ breast cancer cell lines T47D and MCF7. The left of each figure displays the protein, and higher intensity blots represent a higher expression of protein.

Because ER expression remains the same for these controls, inhibiting the 'main' catalytic activity (H3K27me3) of EZH2 does not appear to have an affect in ER+ T47D cells.

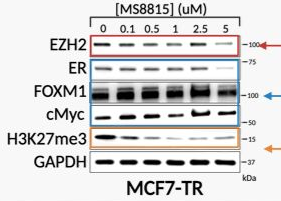
T47D



EZH2 is degradation is facilitated by the MS177 PROTAC (for T47D cells) in a dose-dependant manner

As EZH2 is degraded, ER and the oncogenic transcription factor FOXM1 expression decreases (for T47D cells).

T47D-TR



EZH2 degradation is facilitated by the MS8815 PROTAC (for MCF7 cells).

As EZH2 is degraded, ER and the oncogenic transcription factor FOXM1 expression slightly decreases, though cMyc expression appears to remain constant.

The 'main' catalytic activity (H3K27me3) of EZH2 appears significantly affected by the MS8815 PROTAC in the MCF7 cells.

MCF7-TR

The MS177 PROTAC sensitizes the Tamoxifen resistant (TR) ER+ T47D cells to Tamoxifen in a dose-dependent manner. Further, this PROTAC facilitates degradation of EZH2, correlating with downregulation of estrogen receptor (ER) and oncogenic transcription factors. Finally, treatment of these ER+ breast cancer cells with MS177 correlates with downregulation of estrogen responsive genes and genes correlating with Tamoxifen resistance (data not shown). In contrast, the MS8815 PROTAC appears to inhibit growth of the ER+ TR-MCF7 cells alongside degrading EZH2 and downregulating ER and oncogenic transcription factors, but MS177 does not appear to have an effect in these cell lines. These data suggest possible differences in the canonical H3K27me3 and non-canonical (oncogenic transcription factor and co-activator activation) activities of EZH2 in ER+ breast cancers.

While the MS177 PROTAC appears effective in reducing Tamoxifen resistance in T47D cells, further investigation into the differences that these PROTACs have on different ER+ cell lines will be necessary in developing a therapeutic for treatment of a heterogenous ER+ breast cancer tumors in-vivo.