Evolution of the ability to undergo apoptosis has been associated with many cancers. Overexpression of inhibitors of apoptosis (IAP) proteins is one mechanism by which cancers evade apoptosis. Therefore, IAP proteins have become a promising target for anticancer therapy. Antagonists of IAP proteins have been shown to synergize with TNF receptor family signaling as well as with standard-of-care chemotherapeutics and targeted therapies. However, determinants of sensitivity to single-agent IAP antagonists have not been established. CUDC-427 is a potent, non-redundant IAP antagonist poised for more advanced clinical testing. In the current study, screening of a panel of breast, ovarian, pancreatic, and hematologic cancer cell lines using viability assays in 2D and 3D culture systems identified a group of cell lines that are sensitized to CUDC-427 treatment as a single agent. Mechanism of action studies identified TNFα and XAPF level alterations as putative markers correlated with CUDC-427 sensitivity. These potential predictive markers may inform clinical development of CUDC-427 as monotherapy and in combination with other therapeutic agents.

**CUDC-427 mechanisms of action in breast cancer cell lines**

**CUDC-427 mechanisms of action in lymphoma cell lines**

**CUDC-427 activates caspases**

**Conclusions**

- A group of cell lines were shown to be sensitive to CUDC-427 in both 2D and 3D in vitro culture systems. TNFα sensitizes certain resistant cell lines to CUDC-427 treatment.
- CUDC-427 targets IAP family ligands through the activation of the non-canonical NFκB pathway.
- CUDC-427 sensitivity is induced by IAP antagonism and TNFα activation in both 2D and 3D culture systems.

**Sensitivity of breast, ovarian, pancreatic, and hematologic cancer cell lines to CUDC-427**

A. Growth inhibition sensitivity of CUDC-427 alone and CUDC-427 (100 nM) + TNFα in MDA MB-231 cell lines in 2D culture. B. Growth inhibition of CUDC-427 in a panel of cancer cell lines in 3D culture. C. Sensitivity of hematologic cell lines to CUDC-427 alone or in combination with TNFα as a different indicator. D. Significant correlation of the growth inhibition of CUDC-427 in 2D and 3D culture. Significant correlation observed, P<0.017.

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