Introduction

Evasion from apoptosis is a characteristic of human cancers. The family of Inhibitor of Apoptosis (IAP) proteins plays a pivotal role in apoptosis, proliferation, and signal transduction. Furthermore, mutations, amplifications and chromosomal translocations of IAP genes as well as aberrant expression of IAP proteins are associated with tumor progression, drug resistance and poor prognosis in various malignancies. For example, XIAP is found to be overexpressed in 55% of diffuse large B cell lymphomas (DLBCLs) and significantly linked to poor clinical outcome. Thus, IAP proteins have been promising targets for therapeutic intervention.

CUDC-427 is a potent, oral, monovalent IAP antagonist currently in early-stage clinical testing (NCT01908413). In the present study, the anti-tumor activity of CUDC-427 was evaluated in hematologic malignancies in vitro and in vivo using multiple hematologic cancer cell lines, particularly for B cell lymphomas.

CUDC-427 Induces Apoptosis in Sensitive DLBCL Cell Lines

A. Western blot analysis of IAPs and their downstream markers in WSU-DLCL2 and Karpas 422 cell lines treated with CUDC-427 for 6 or 22 hours, as indicated. Tubulin was used as a loading control.
B. Caspase 3/7 induction as determined by Caspase-Glo 3/7 assay in WSU-DLCL2 and Karpas 422 cell lines treated with CUDC-427 for 24 hours. Values indicated are normalized to DMSO control and error bars represent SD.
C. Caspase 3/7, 8 and 9 induction as determined by Caspase-Glo 3/7, Caspase-Glo 8 and Caspase-Glo 9 assays in WSU-DLCL2 cell lines treated with CUDC-427 for 24 hours. Values indicated are normalized to DMSO control and error bars represent SD.

Mouse Stromal Cells Sensitize Karpas 422 DLBCL Cells to CUDC-427 Treatment

A. Co-culture of Karpas 422 DLBCL cells with mouse stromal cells (MSC) enhances CUDC-427-induced caspase 3/7 activation. CUDC-427-induced caspase 3/7 activation, as determined by Caspase-Glo 3/7 assay, in Karpas 422 cells, MSC, and co-culture of Karpas 422 and MSC after 24-hour incubation. Values indicated are normalized to DMSO control and error bars represent SD.
B. MSC co-culture does not increase human or mouse TNFα secretion from Karpas 422 cells or MSC respectively. Human and mouse TNFα levels in the culture media of Karpas 422 cells, co-culture of karpas 422 and MSC, or MSC alone were detected by ELISA. Values indicated are normalized to DMSO control and error bars represent SD. Mouse TNFα, human and mouse TRAIL were not detectable in all culture conditions tested (data not shown).

Conclusions

- The anti-tumor activity of CUDC-427 was evaluated against a large panel of human cell lines of hematologic cancer origins. This screen identified DLBCL cell lines to be the most sensitive cell type.
- The addition of TNFα family ligands significantly increased the sensitivity to single-agent CUDC-427 treatment in insensitive DLBCL cell lines.
- CUDC-427 activated caspases in the sensitive DLBCL cell line (WSU-DLCL2) but not in the resistant cell line (Karpas 422). However, the presence of stromal cells in the culture sensitized the Karpas 422 DLBCL cells to CUDC-427 treatment as measured by caspase 3/7 activation.
- CUDC-427 induced 94% tumor growth inhibition in WSU-DLCL2 xenograft model, and tumor regression in Karpas 422 xenograft model. CUDC-427 treatment also induced tumor stasis in the fast growing A20 B cell lymphoma syngeneic model, which may be partly due to high levels of TRAIL in the tumor tissue in this model.