Voruciclib, a clinical stage CDK inhibitor sensitizes triple negative breast cancer xenografts to proteasome inhibition

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Abstract

Triple negative breast cancer (TNBC) is a highly heterogeneous disease that is notoriously challenging to treat with standard chemotherapy options, and it is therefore an area of intense focus for discovery of novel effective combination therapies. Here we used a previously described technology platform called CIVO, which enables assessment of multiple drugs and drug combinations simultaneously in living tumors, to identify drug combinations that result in synergistic anti-tumor activity in the HCC1187 model of TNBC. Our study aimed to identify agents that result in induction of pro-survival regulators such as MCL1 - a direct target of CDKs, and therefore are good candidates to combine with voruciclib, a novel clinical stage oral CDK inhibitor with potent activity against CDK9 among others. In a CIVO screen with rationally selected drugs, bortezomib, a proteasome Inhibitor, acutely induced the highest localized expression of MCL1 which is known to contribute to resistance against this class of compounds. Combining voruciclib and bortezomib led to robust localized anti-tumor activity as measured by cleaved caspase 3 positive apoptotic cells. In contrast, exposure to either voruciclib or bortezomib as single agents showed little anti-tumor activity. Importantly, results obtained with CIVO accurately predicted the outcome of systemic chemotherapy studies where tumor regression or sludge was induced by combining voruciclib with either bortezomib or a next-generation oral proteasome inhibitor, MLN2238. No significant impact on tumor progression was observed in xenografted subcutis treated with either single agent. The ability of TNBC cell lines to withstand stresses such as chemotherapy may be due in part to accumulation of anti-apoptotic proteins MCL1, XIAP and activation of other adaptive survival pathways such as the unfolded protein (UPR) and endoplasmic reticulum (ER) stress responses. As observed in previous reports, exposure of HCC1187 cells to bortezomib alone led to an increase in two markers of the cyto-protective arm of the UPR/ER stress pathway: XBP1-s and GRP78/BIP. Consistent with the possibility that voruciclib impedes the cytoprotective UPR/ER stress response induced by bortezomib, exposure to the drug combination substantially reduced protein expression of both XBP1-s and GRP78. Voruciclib neutralized upregulation of these same proteins by the classic ER stress inducing agent bryostatin. Bortezomib-induced MCL1 and XIAP accumulation were also blocked by voruciclib, consistent with its CDK9 inhibitory properties, with concomitant induction of c-chap. These studies provide a foundation for further investigation of anti-cancer agents that induce UPR/ER stress responses in combination with voruciclib for treating TNBC patients.

CIVO technology identifies proteasome inhibitors as agents that lead to MCL1 accumulation in TNBC model

The CIVO Platform

The CIVO platform consists of 2 components:
(1) A hand-held device enabling simultaneous delivery of micro doses of drugs into living tumors
- A fluorescent tracking marker (FTM) is co-injected through the needle to demarcate injection sites
- Tumor responses are assessed ex vivo with immunohistochemical staining for biomarkers, following injection at predefined time points
(2) An automated custom image analysis platform – CIVO Analyzer:
- High resolution whole-slide scanning is used to capture images from each histological section
- A representative tumor response at a single site is shown: Nuclear DAPI stain; FTM (green) drug specific biomarker (red) resulting from the injection site
- Results integrated into custom CIVO analysis which classifies cells within each region of interest as bioactive-apoptotic (green dots), or non-apoptotic (red dots).

Voruciclib blocks pro-survival pathways that contribute to resistance mechanisms

Voruciclib – a CDK inhibitor with a unique target profile that inhibits CDK9 with high potency

Voruciclib downregulates CDK9 target, MCL1 in multiple xenograft models

Voruciclib combined with bortezomib enhances apoptosis and impedes HCC1187 tumor growth

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Summary

- Voruciclib is a clinical-stage, oral CDK9/6/4/1 selective inhibitor
- Tumor growth inhibition of TNBC xenografts by voruciclib + proteasome inhibitors is superior to that of the respective single agents
- Voruciclib blocks resistance-inducing pro-survival regulators thereby sensitizing tumor cells to proteasome inhibition and cell death

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Acknowledgments to Emily Bieme and Kate Gilespie at Presage Histology Core, Micah Ellison and Sheng You for software and database support, Chaithil Dixon for imunostain support.