#2835

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

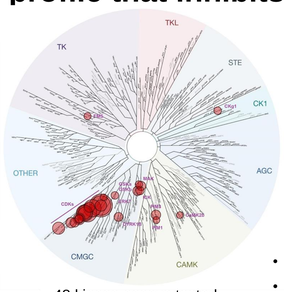
Joyoti Dey[1], Joseph Casalini[1], Marc Grenley[1], William Kerwin[1], Angela Merrell[1], Sally Ditzler[1], Connor Burns[1],Thomas Deckwerth[1], Jason Frazier[1], Richard Klinghoffer[1]

[1]Presage Biosciences, Seattle, WA

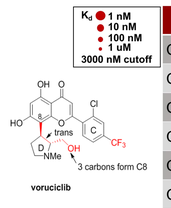
Abstract

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 CDK9, 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 drug efficacy studies where tumor regression or stasis were 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 subjects treated with either single agent. The ability of TNBC cells to withstand stressors 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: XBP-1s and GRP-78/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 XBP-1s and GRP-78. Voruciclib neutralized upregulation of these same proteins by the classic ER stress inducing agent tunicamycin. Bortezomib-induced MCL1 and XIAP accumulation were also blocked by voruciclib, consistent with its CDK9 inhibitory properties, with concomitant induction of cPARP. 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.

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



48 kinase assays tested
27 interactions mapped



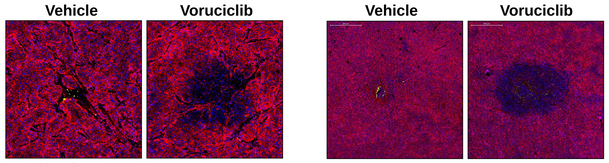
Target	K_i (nM)
CDK9/cyc T2	0.626
CDK9/cyc T1	1.68
CDK6/cyc D1	2.92
CDK4/cyc D1	3.96
CDK1/cyc B	5.4
CDK1/cyc A	9.1

- Low nanomolar potency against CDK9, 6, 4 and 1
- Two Phase 1 single agent safety studies completed
- Phase 1b combination trial in metastatic melanoma

Voruciclib downregulates CDK9 target, MCL1 in multiple xenograft models

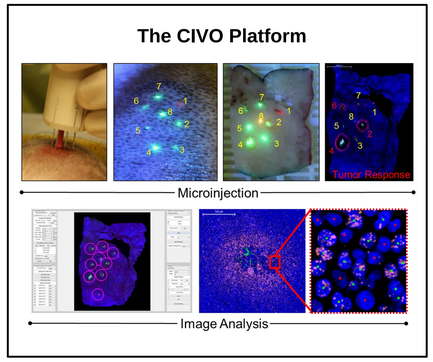
Solid Tumor
HCC1187 TNBC model

Hematogical Tumor
RIVA DLBCL model



CIVO technology enabled microinjection of voruciclib in multiple xenograft models show localized downregulation of MCL1 via immunohistochemistry (IHC, t = 6 hours).

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



The CIVO platform consists of 2 components:

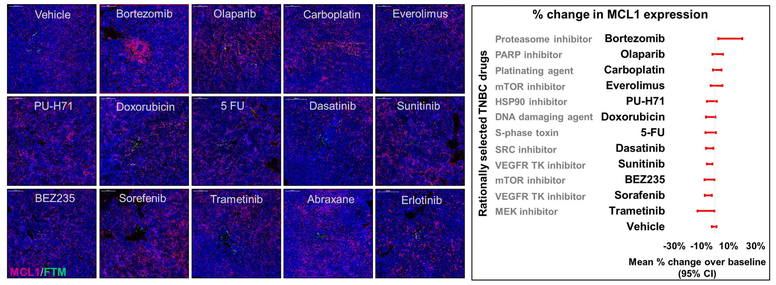
(1) A hand-held device enabling simultaneous delivery of micro doses of drugs into living tumors

- A fluorescent tracking marker (FTM) is co-injected through each needle to demarcate injection sites
- Tumor responses are assessed *ex vivo* with immunohistochemical staining for biomarkers, following resection at predetermined time points.

(2) An automated custom image analysis platform – CIVO Analyzer.

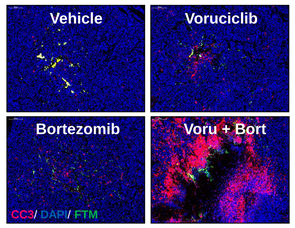
- High resolution whole-slide scanning is used to capture images from each histological section
- A representative tumor response at a single site is shown. Nuclei: DAPI (blue); FTM (green); drug-specific biomarker (red) radiating from the injection site.
- Resulting images are processed by CIVO Analyzer, which classifies cells within each region of interest as biomarker-positive (green dots) or negative (red dots).

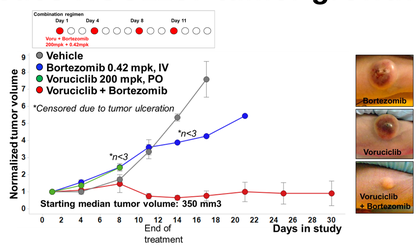
A screen with the CIVO platform using rationally selected TNBC drugs



(Left) CIVO screen in the HCC1187 TNBC xenograft model with rationally selected TNBC drugs demonstrates by IHC, that localized MCL1 upregulation induced by proteasome inhibitor bortezomib is the most pronounced compared to other drugs tested (t = 24 hours). (Right) Plot shows % change in MCL1 expression over baseline, induced by each of the microinjected agents across n=3 tumors.

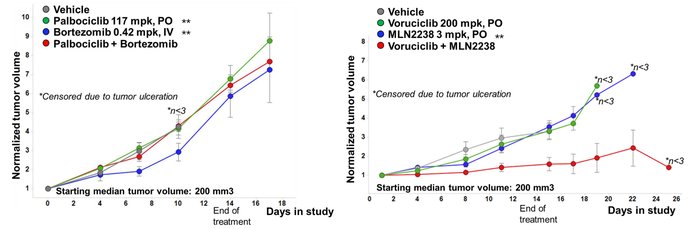
Voruciclib combined with bortezomib enhances apoptosis and impedes HCC1187 tumor growth





(Left) HCC1187 xenograft tumors were injected using the CIVO device with vehicle, voruciclib, bortezomib and a combination thereof, in the same tumor and resected 24 hours post injection. Representative images show apoptotic response via cleaved caspase 3 IHC; injection sites are demarcated by FTM. (Right) HCC1187 xenografts were systemically treated with the same drugs and drug combination. Drug efficacies were assessed via tumor volume measurements twice weekly and normalized to starting volumes.

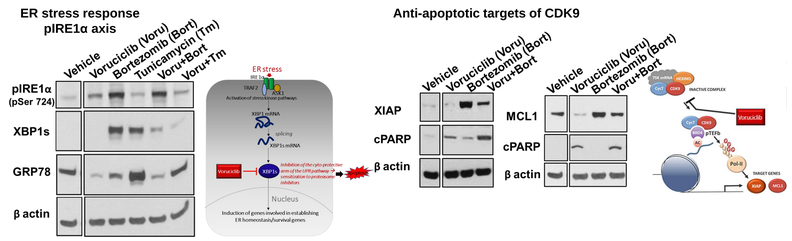
Combination effect is specific to voruciclib, but includes other proteasome inhibitors



HCC1187 xenografts were systemically treated with vehicle, CDK4/6 specific inhibitor palbociclib QD x 14, bortezomib twice weekly x 2 wk (left) or voruciclib 5 times per wk x 2 wk, MLN2238 twice weekly x 2 wk (right), and respective combinations. Drug efficacies were assessed via tumor volume measurements twice weekly and normalized to starting volumes.

*** MLN2238, Palbociclib and Bortezomib were purchased from commercial vendors*

Voruciclib blocks pro-survival pathways that contribute to resistance mechanisms



(Left) Western blot analyses of HCC1187 lysates at 24 hours post treatment show, voruciclib downregulates bortezomib or tunicamycin induced XBP1s and GRP78 (cyto-protective components of the ER stress/UPR pathway) despite activation of IRE1α upstream in the axis and (right) CDK9 targets XIAP (24 hrs) and MCL1 (6 hrs).

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**

Contact Information:
Nathan Caffo (President) businessdevelopment@presagebio.com
Richard Klinghoffer (CSO)

Acknowledgments to Emily Bierne and Kate Gillespie at Presage Histology Core, Micah Ellison and Sheng You for software and database support, Chantel Dixon for vivarium support.