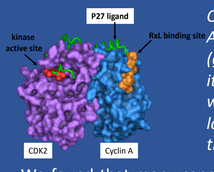
Mechanisms responsible for hypersensitivity of small cell lung cancers to novel Cyclin A/B RxL macrocyclic peptide inhibitors

Shilpa Singh[1], Yavuz T. Durmaz[1], Catherine E. Gleason[2], Evelyn W. Wang[2], Rajinder Singh[2], David J. Earp[2], Pablo D. Garcia[2], Matthew G. Oser[1,3]
[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts.
[2]Circle Pharma Inc., 681 Gateway Blvd, South San Francisco, CA 94080
[3]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

Abstract no. 1559

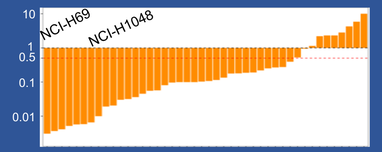
INTRODUCTION

- Cyclins regulate the progression of cells through the cell cycle by binding and activating cyclin-dependent kinases (CDKs) and selective targeting of Cyclins and/or their CDKs are promising therapeutic strategies for various cancer types. Most cancer-directed targeted therapies are developed using small molecule inhibitors that require a druggable binding pocket on the target of interest. Macrocyclic peptides can selectively block protein-protein interactions required for function and hence can be used to target previously undruggable proteins. Using a structure-guided approach, we developed cell-permeable macrocyclic peptides that block the ability of Cyclin A and/or Cyclin B, to bind RxL motifs on their substrates thereby inhibiting Cyclin activity.



Crystal structure of Cyclin A/CDK2 bound to p27. p27 (green) binds to Cyclin A via its RxL domain (orange) which facilitates proper localization of p27 within the active site of CDK2(red).

- We found that many cancer cell lines with RB1/E2F dysregulation were highly sensitive to Cyclin macrocyclic peptides, which includes most small cell lung cancer (SCLC) cell lines with *RB1* and *TP53* inactivation.

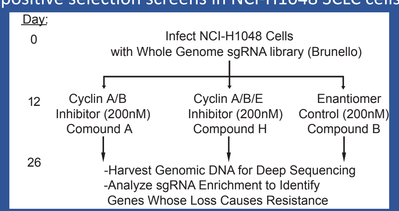


Waterfall plots of 44 SCLC cell lines treated with Compound A (as assessed by GI_{50}).

- CRISPR/Cas9 positive selection resistance screens were performed to identify genes responsible for hypersensitivity of SCLC cell lines to Cyclin A/B RxL inhibitors.

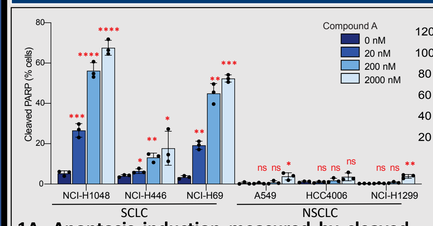
METHODS

Schema for the CRISPR/Cas9 genome-wide positive selection screens in NCI-H1048 SCLC cells.

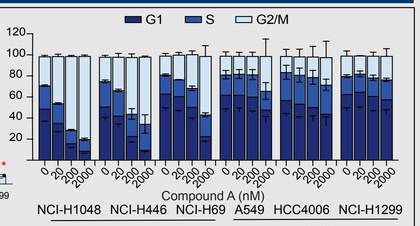


RESULTS

Figure 1. Small Cell Lung Cancer Cell Lines (SCLC) are highly sensitive to Cyclin A/B inhibitors which induce mitotic arrest and apoptosis



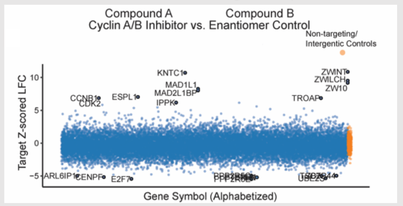
1A. Apoptosis induction measured by cleaved PARP FACS analysis in different lung cancer cell lines treated with Compound A (Cyclin A/B inhibitor) at concentrations indicated for 72 hours.

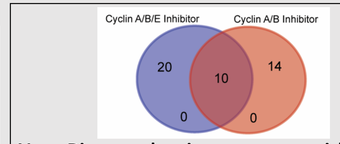


1B. Cell cycle distribution measure by PI FACS analysis of lung cancer cell lines treated with Compound A (Cyclin A/B inhibitor) at concentrations indicated for 24 hours.

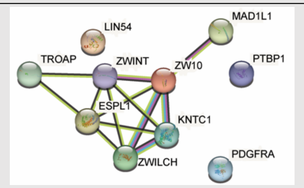
Figure 2. Genome-Wide CRISPR/Cas9 Positive Selection Screen Reveals SAC Activation as a dominant mechanism of cell killing by Cyclin A/B inhibitor

Apron analysis comparing the end timepoint for each drug condition indicated vs. Compound A enantiomer control. n=2 biological replicates.

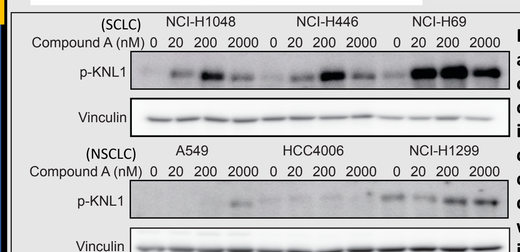




Venn Diagram showing common enrichment hits between different treatments.

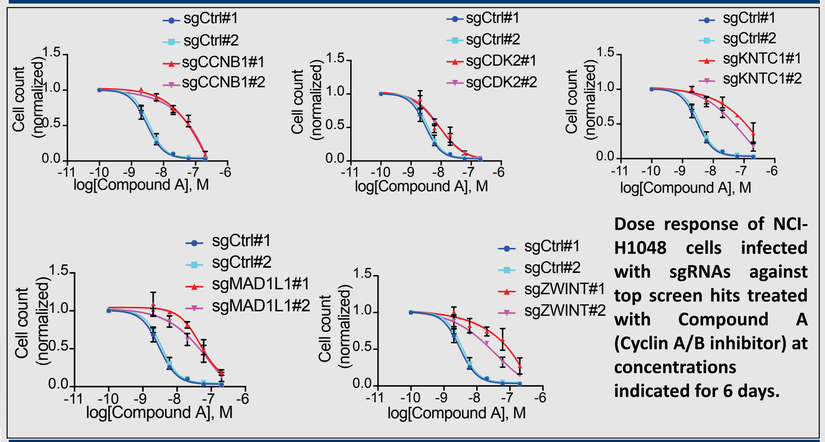


STRING analysis of common hits between Compound A and Compound H



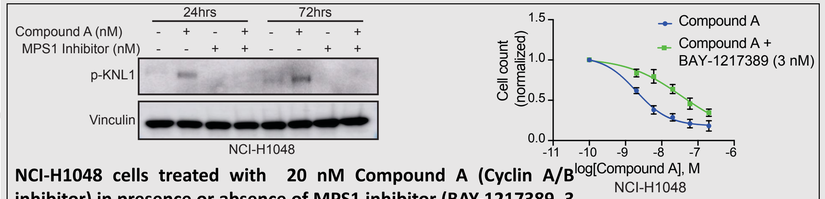
Phospho-KNL1 immunoblot analysis as a surrogate for spindle assembly checkpoint (SAC) activation in SCLC cells compared to relatively insensitive non-small cell lung cancer cell lines (NSCLC) selected as controls from cell growth inhibition data (Abstract 1560) and treated with Compound A at concentrations indicated for 24 hours.

Figure 3. CRISPR inactivation of selected screen hits caused resistance to Cyclin A/B RxL Peptide Inhibitors



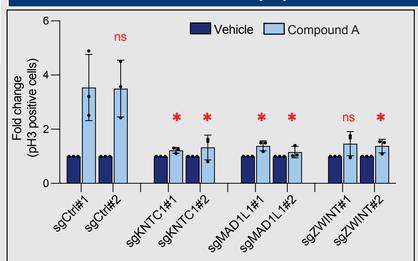
Dose response of NCI-H1048 cells infected with sgRNAs against top screen hits treated with Compound A (Cyclin A/B inhibitor) at concentrations indicated for 6 days.

Figure 4. Spindle Assembly Checkpoint (SAC) Activation by MPS1 is Necessary for Cyclin A/B inhibitors to Block Proliferation in SCLC Cell Lines

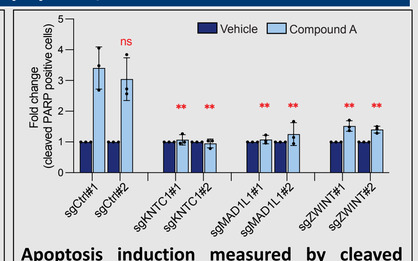


NCI-H1048 cells treated with 20 nM Compound A (Cyclin A/B inhibitor) in presence or absence of MPS1 inhibitor (BAY-1217389, 3 nM) for 24 hrs (left) and dose response at 72 hrs (right).

Figure 5. Genetic disruption of the SAC complex significantly decreases mitotic arrest and apoptosis caused by Cyclin A/B Inhibitors

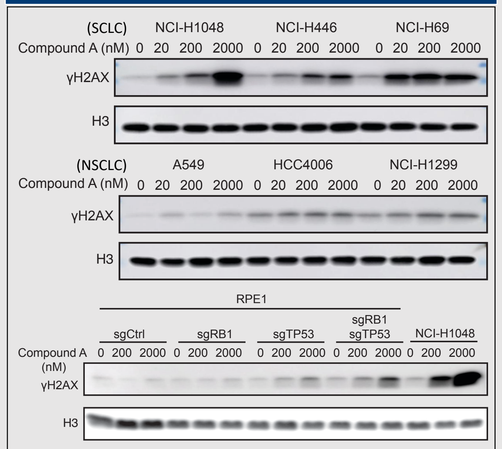


FACS analysis of phosphoserine10-H3 in NCI-H1048 cells treated with Compound A (Cyclin A/B inhibitor, 20 nM) for 24 hours.



Apoptosis induction measured by cleaved PARP FACS analysis in NCI-H1048 cells treated with Compound A (Cyclin A/B inhibitor, 20 nM) for 24 hours.

Figure 6. Cyclin A/B Inhibition Promotes DNA Damage Preferentially in Sensitive SCLC Cell Lines with RB1/TP53 Inactivation



γH2AX immunoblot analysis as a surrogate for DNA damage accumulation of SCLC/NSCLC lung cancer cell lines and RPE1 (non-transformed) cells treated with Compound A (Cyclin A/B inhibitor), 72 hrs

CONCLUSION

- SCLC cell lines are highly sensitive to cyclin A/B RxL peptide inhibitors where Cyclin A/B RxL inhibitors induce DNA damage and mitotic arrest leading to apoptosis.
- Genome-wide CRISPR knock out screen revealed that MAD1 and the ROD-Zwilch-Zw10 (RZZ) complex of the mitotic spindle assembly checkpoint (SAC), which is regulated by the kinase Mps1, is required for Cyclin A/B inhibitors to induce apoptosis.
- Chemical inhibition of Mps1 caused Cyclin A/B inhibitor resistance demonstrating that SAC activation by Mps1 is required for the ability of Cyclin A/B inhibitors to induce apoptosis.
- Overall, selective inhibition of cyclins with RxL macrocyclic peptides offers a mechanistically distinct mode of action to inhibit Cyclin/CDKs.