



MCL

## AACR 2017 | Poster 4051/21 – Combination efficacy of vorinostat (HDAC inhibitor) and palbociclib (CDK-4/6 dual inhibitor) against therapy-resistant Mantle Cell Lymphoma

 Terri Penfold | Apr 13, 2017

**At the American Association for Cancer Research (AACR) annual meeting in Washington, DC, USA, on Tuesday 4<sup>th</sup> April, a poster session titled “Combination Therapies and Approaches to Sensitize Cancer Cells to Drugs” took place.**


One of the posters on display (4051 / 21) was titled “Combination efficacy of HDAC inhibitor vorinostat and CDK-4/6 dual inhibitor palbociclib against therapy-resistant mantle cell lymphoma” by Nagendra K. Chaturvedi from the University of Nebraska Medical Center, Omaha, NE, and colleagues.

This group analyzed the efficacy of vorinostat as a single agent and in combination with palbociclib on MCL cell growth, survival, and underlying molecular mechanism(s) using different cell lines (Granta 519, Jeko-1, and JVM-2). Therapy-resistant MCL cell lines were also used, derived from Granta-519 (GRL, GRK, and GRR).

### Key Highlights:

- Both vorinostat and palbociclib, as single agents or combined, significantly suppressed cell growth and induced apoptosis in therapy-resistant and other MCL lines
- Combining vorinostat and palbociclib significantly inhibited the activation of phosphorylated-retinoblastoma protein (a key component of cell cycle regulation) and increased expression of acetylated-Histone H3
- Expression of Cyclin D1 and Bcl-2 proteins were downregulated by these inhibitors

The poster concluded by stating that these results indicate that combination of vorinostat and palbociclib demonstrated significant synergistic activity in MCL by targeting associated pathways/molecules. The authors stressed that this combination should be evaluated in further preclinical studies in order for translation to the clinic.



## Combination efficacy of HDAC inhibitor vorinostat and CDK-4/6 dual inhibitor palbociclib against therapy-resistant mantle cell lymphoma

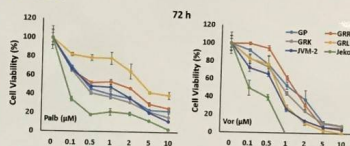
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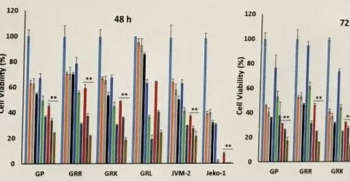
**ABSTRACT**

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma accounting for about 7% of all non-Hodgkins lymphoma. While multiple therapy regimens are available to treat MCL patients, ultimately relapse from therapy-resistant MCL, making MCL carry the worst prognosis of all B cell lymphomas. Emerging evidence suggest that dysregulated histone deacetylases (HDACs) and the key molecules of cell cycle regulator cyclin-dependent kinases (CDKs) have been shown to be commonly associated with many lymphomas including MCL, and are considered as promising targets for relapsed lymphoma therapy. Several inhibitors of these target pathways/molecules are in clinical trials as monotherapies or in combination with other anticancer agents. Therefore, in this study, we investigated the single agent and combination efficacies of the HDACs inhibitor vorinostat, and CDK-4/6 dual inhibitor palbociclib on MCL cell growth/survival and underlying molecular mechanism(s) using different MCL cell lines (Granta 519, Jeko-1 and JVM-2) including therapy-resistant MCL cell lines derived from Granta-519 (GRL, GRK and GRR). We used MTT assay to measure the survival and proliferation, agar colony formation assay to determine the effects of these inhibitors on the anchorage independent growth, Annexin-V analyses to examine the cells undergoing apoptosis following treatments and western blot analyses to determine the expression levels of target molecules including pRb, histone acetylation, cyclin-D1 and Bcl-2. The concentration of these inhibitors used in this study were similar to the published literature including sub-IC<sub>50</sub> levels determined in our laboratory. Our results showed that both inhibitors as single agents or combined, significantly suppressed the cell growth, and induced apoptosis in therapy-resistant and other MCL lines *in vitro*. The single agent and combined anti-lymphoma efficacies of these inhibitors were further confirmed with the colony formation ability of the MCL cells grown in 0.3% agar semisolid media. In addition, combination of vorinostat and palbociclib treatment significantly inhibited the activation of phosphorylated-retinoblastoma (pRb) protein, a key molecule of cell cycle and increased the expression of acetylated-Histone H3 (H3-Ac) as determined by western blot analyses. Subsequently, the expression of Cyclin D1 (proliferation, MCL hallmark) and anti-apoptotic Bcl-2 proteins were also downregulated by these inhibitors. Together, our findings suggest that combination of vorinostat and palbociclib showed a significant synergistic anti-MCL activity by targeting associated pathways/molecules. This targeted approach warrants further preclinical evaluation for translation to the clinic.

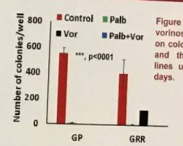
**RESULTS**



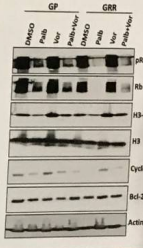
**Figure 1:** Single agent efficacies of palbociclib (Palb) and vorinostat (Vor) on MCL cell growth in a dose-dependent manner.



**Figure 2:** Combination efficacy of vorinostat and palbociclib on MCL cell growth using MTT assay. The L, M and H indicate low (0.5 µM), medium (1 µM) and high (2 µM) concentrations of each inhibitor, respectively. \*\*, p<0.01.



**Figure 4:** Combination efficacy of vorinostat (1 µM) and palbociclib (1 µM) on colony forming ability in GP parental and therapy-resistant GRR MCL cell lines using 0.3% semi-solid Agar for 15 days.



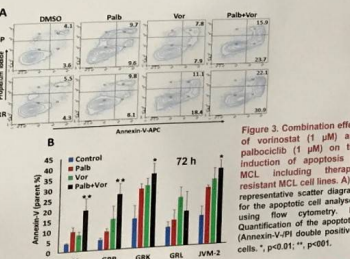
**Figure 5:** Combination effect of vorinostat and palbociclib on the expression of target pathways and associated proteins in MCL. GP parental and therapy-resistant GRR MCL cell lines were treated with 1 µM concentration of each inhibitor alone or combined for 24 hours and the expression of associated pathways/molecules were determined using western blot analyses. Actin was used as a loading control in these experiments.

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**METHODOLOGY**

To determine the *in vitro* combined efficacies of selected inhibitors, the MCL cell lines [Granta 519 (GP), Jeko-1 and JVM-2] including therapy-resistant MCL cell lines derived from Granta 519 (GRL, GRK and GRR) were treated with HDAC inhibitor vorinostat and CDK-4/6 dual inhibitor palbociclib alone or combined in a dose-dependent manner for 48 and 72 hours. Following treatments, the single agent and combined efficacies of inhibitors were accessed using MTT, Annexin-V/PI on cell growth, apoptosis and colony formation. Further, their efficacies on targeting the associated pathways/molecules including the expression levels of phosphorylated retinoblastoma (pRb), histone H3 acetylation (H3-Ac), Cyclin-D1 (MCL proliferation hallmark) and anti-apoptotic Bcl-2 proteins were investigated using western blot analyses. Each experiment was repeated 2-3 times and the mean and standard error values of all experiments were calculated. The significance of differences (p-value) was calculated using independent Student t-tests and the p-values less than 0.05 considered significant.

**Figure 3:** Combination effect of vorinostat (1 µM) and palbociclib (1 µM) on the induction of apoptosis in MCL, including therapy-resistant MCL cell lines. A) A representative scatter diagram for the apoptotic cell analyses using flow cytometry. B) Quantification of the apoptotic (Annexin-V-PI double positive) cells. \*\*, p<0.01; \*\*\*, p<0.001.




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**SUMMARY & CONCLUSIONS**

- HDAC inhibitor vorinostat and CDK-4/6 dual inhibitor palbociclib suppressed cell growth/survival in MCL cells including therapy-resistant MCL cells *in vitro*.
- Combination of these inhibitors significantly decreased cell growth and induced apoptosis in MCL cells including therapy-resistant MCL cells.
- These inhibitors downregulated the expression of key molecules of their target pathway in MCL.
- Thus, our studies with the combined efficacy of inhibitors against MCL shown *in vitro* warrants further preclinical evaluation of this therapeutic approach for translation to the clinic.

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**Reference:**

1. Chaturvedi N.K, et al. Combination efficacy of HDAC inhibitor vorinostat and CDK-4/6 dual inhibitor palbociclib against therapy-resistant mantle cell lymphoma [Poster]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2017 Apr 1-5; Washington, DC. Philadelphia (PA): AACR; 2017. Poster nr [4051 / 21].

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