ON 123300, an Orally Administered Novel CDK4/6 + ARK5 Inhibitor, Exhibits Potent Antitumor Activity In Vivo: Comparative Studies with Palbociclib

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INTRODUCTION

- The overexpression of cyclin-dependent kinases 4/6 (CDK4/6) is known to cause cell cycle dysregulation in certain cancer types, making these cell cycle kinases attractive targets for pharmacological inhibition. The effectiveness of first-generation non-selective cyclin-dependent kinases, such as roscovitine and flavopiridol, was hampered by toxicities, leading to the development of second-generation compounds like IBRANCE®/Palbociclib that specifically inhibit CDK4 and 6.

- ON 123300 is a third-generation potent CDK4/6 inhibitor that also inhibits ARK5 with low nanomolar potency and has the potential to improve upon second-generation compounds. Previous studies have demonstrated the inhibitory effect of single-agent ON 123300 in various pre-clinical cancer models of MM and leukemia. [1 & 2]

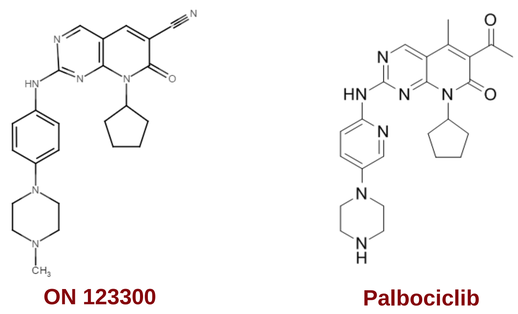
- In this study, we investigated the comparative therapeutic potential of ON 123300 as an oral anticancer agent and a second-generation inhibitor, Palbociclib, in xenografted Rb+ve mouse models.

METHODS

- MDA-MB-435S xenografted mice were treated once a day for 21 days with ON 123300 (125mg/kg) or Palbociclib (125mg/kg). Tumor volumes were measured and peripheral blood was gathered to evaluate the effects on hematological parameters. Separately, Western blot analyses were performed to determine the effect of CDK4/6 inhibition on p-Rb following intra-tumoral treatment with ON 123300 (2.5µM) or Palbociclib (2.5µM).

RESULTS

Figure 1. Chemical structure of ON 123300 and Palbociclib:



ON 123300 **Palbociclib**

Figure 2. Treatment with ON 123300 or Palbociclib exhibit comparable anticancer activity in Rb+ve *in vitro* cancer models:

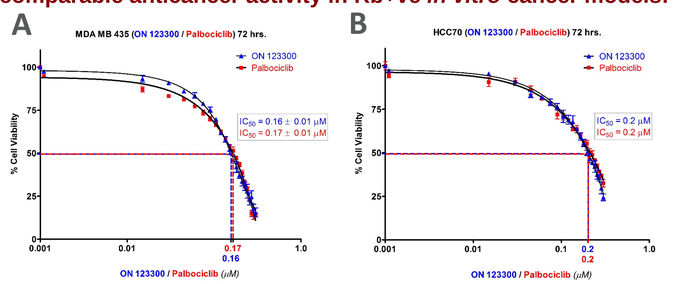


Fig. 2: (A & B) The cell viability assay data indicate significant inhibition of MDA-MB-435S and HCC70 Rb+ve cancer cell lines after treatment with various concentrations of ON 123300 (0.15-0.30 µM) and Palbociclib (0.15-0.30 µM).

Figure 3. Treatment with ON 123300 inhibits expression of p-Rb in MDA-MB-435S Rb+ve cancer cells :

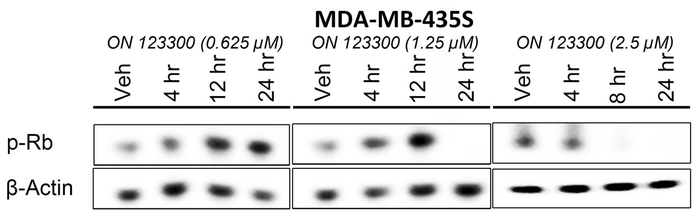


Fig. 3: MDA-MB-435S cells were treated with various concentrations of ON 123300 (0.625-2.5 µM) for multiple time points (4-24 hrs.). The protein expression data exhibit a dose- and time-dependent decrease in pRb levels after treatment with varying concentrations of ON 123300.

Figure 4. Treatment with ON 123300 or Palbociclib exhibits similar anti-tumor activity *in vivo*:

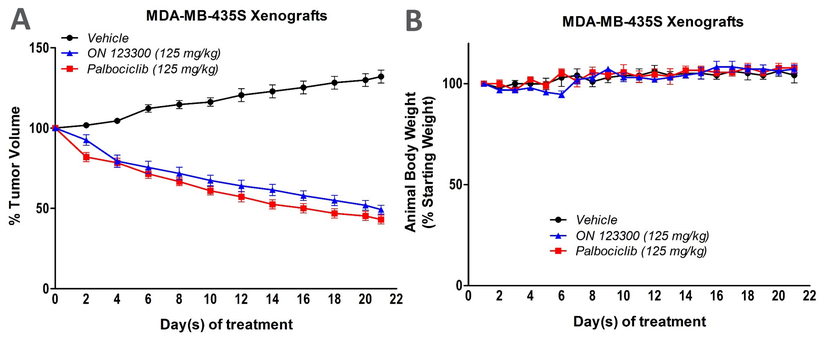


Fig 4: MDA-MB-435S xenografted mice were treated QD with ON 123300 (125 mg/kg) or Palbociclib (125 mg/kg) for 21 days. **(A)** The tumor volume data indicate comparable and significant anti-tumor activity that treatment with ON 123300 or Palbociclib. **(B)** Animal body weight does not show any significant change after treatment with ON 123300 or Palbociclib.

Figure 5. Treatment with ON 123300 exhibits reduced suppression of neutrophils in comparison to Palbociclib in mouse xenografts:

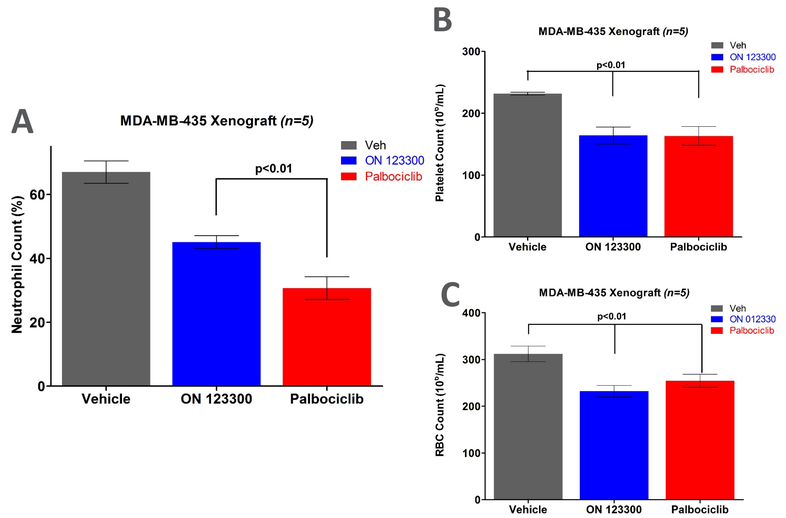


Fig. 5: MDA-MB-435S xenografted mice were treated QD with ON 123300 (125 mg/kg) or Palbociclib (125 mg/kg) for 21 days. **(A)** The blood cell count data indicate that treatment with ON 123300 exhibits significantly improved neutrophil count when compared to Palbociclib in xenografted mice after treatment for 21 days. **(B & C)** Platelet and RBC counts show similar degree of inhibition after treatment with ON 123300 or Palbociclib. *Veh - Vehicle.*

Figure 6. Intra-tumoral treatment with ON 123300 or Palbociclib significantly inhibits expression of pRb in tumor tissue:

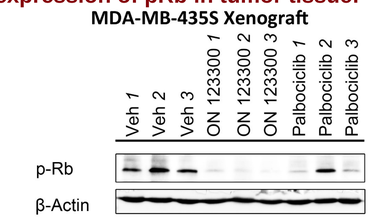


Fig 6: The protein expression data shows that intra-tumoral treatment with ON 123300 or Palbociclib mediated significant reduction in expression of pRb in xenografted tumor tissue. *Veh - Vehicle and Palbo - Palbociclib.*

CONCLUSION

- Our *in vitro* and xenograft data indicates that ON 123300, a third-generation CDK4/6 inhibitor, is as effective as Palbociclib in an Rb+ve xenograft model.
- In addition, this study also suggests that ON 123300 may have the added advantage of reduced neutropenia when compared to Palbociclib.
- Prior preclinical data suggest that ON 123300 may be efficacious in Rb-ve tumors, where second-generation compounds have diminished single-agent activity, and our ongoing studies are aimed at further characterizing the in vivo activity of ON 123300 in this setting.

ACKNOWLEDGEMENTS

- This study was supported by grants from Onconova Therapeutics Inc. and the work was done at Dept. of Pharm. Sciences, Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, NY 11201.

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