Briciclib and its oral derivative (ON 013100) exhibit comparable anticancer activity in various preclinical cancer models

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INTRODUCTION

- Eukaryotic translation initiation factor 4E (eIF4E) is a master regulator that controls translation of mRNA in mammalian cells. eIF4E is potent proto-oncogene that promotes translation of several genes essential for cellular proliferation (cyclin D1, c-Myc, mTOR), survival (Akt, survivin), angiogenesis (VEGF), and metastasis (MMP9)[1].
- Overexpression of eIF4E has been observed in almost all major groups of cancers and has been shown to induce increased expression of cyclin D1 and c-Myc[2].
- In this study we investigated and compared the anticancer activity of Briciclib (ON 013105), a novel investigational eIF4E-selective inhibitor, to its precursor ON 013100. Briciclib, a water-soluble derivative of ON 013100, is designed for intravenous therapy whereas ON 013100 is a small molecule inhibitor that can be administered orally. We determined the susceptibility of various breast, mantle cell leukemia (MCL), gastric, and esophageal cancer cell lines to treatment with Briciclib or ON 013100. In addition, we also investigated the effect of Briciclib or ON 013100 on expression of markers associated with eIF4E activity (cyclin D1 and c-Myc) and apoptosis (P53 and Cleaved Caspase 3).

METHODS

- MTT cell viability assay, Western blot analysis, and ELISA were done to evaluate cellular viability, survival, and protein expression levels.

RESULTS

Figure 1. Treatment with Briciclib or ON 013100 significantly inhibits cell viability of various cancer cell lines:

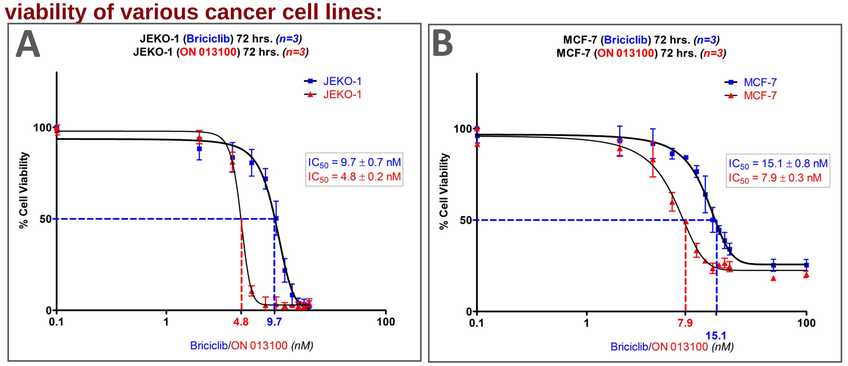


Fig. 1: (A & B) The cell viability assay data indicates significant inhibition of JEKO-1 MCL, and MCF-7 breast cancer cell lines after treatment with various concentrations of Briciclib (2-100 nM) and ON 013100 (2-100 nM).

Figure 2. Treatment with Briciclib or ON 013100 significantly inhibits survival of cancer cells lines:

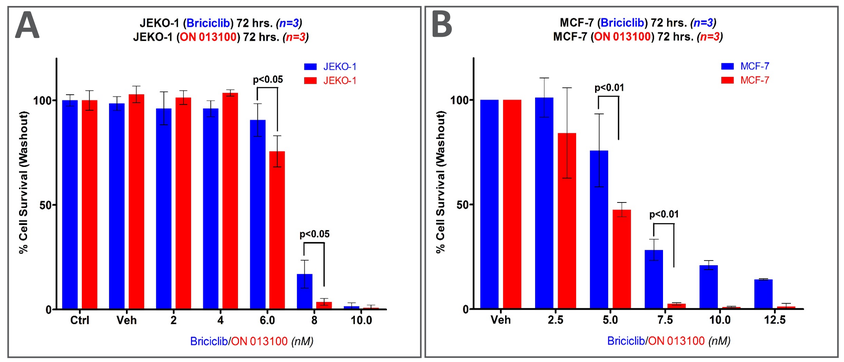
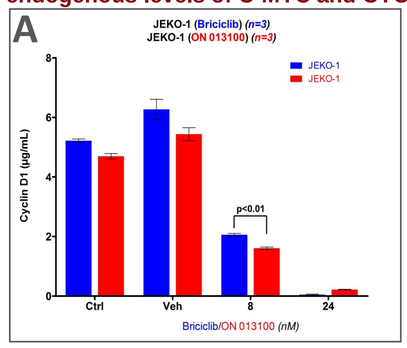


Fig. 2: (A) MCL cancer cells (JEKO-1 and *MINO - data not shown*) were treated with varying concentrations of Briciclib or ON 013100 (2-10 nM) for 72 hrs. and subsequently incubated in drug free medium for 72 hrs. Cell survival was determined by counting cells with a hemocytometer. **(B)** Breast cancer cell lines (MCF-7 and *MDA MB 231 - data not shown*) were treated with Bricicclib or ON 013100 (2-12.5 nM) for 24 hrs. and then incubated in drug free medium for 7 days. Cell survival was determined by counting colonies stained with crystal violet. The data indicates significant inhibition of cell survival for both MCL and breast cancer cells after treatment with Briciclib or ON 013100.

Figure 3. Treatment with Briciclib or ON 013100 reduces endogenous levels of C-MYC and CYCLIN D1:



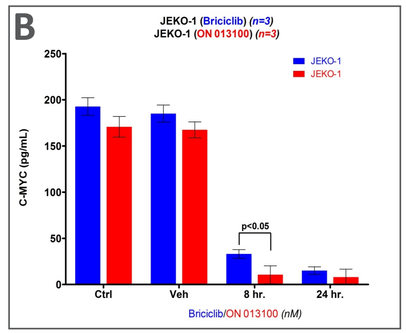


Fig. 3: (A & B) JEKO-1 MCL cells were treated with Briciclib or ON 013100 (0.5 µM) for 8 hrs. and 24 hrs. Subsequently, cell lysates were made and ELISA was done to determine endogenous levels of CYCLIN D1 and C-MYC proteins. The ELISA data indicates a significant reduction in endogenous levels of CYCLIN D1 and C-MYC after treatment with Briciclib or ON 013100.

Table 1. IC_{50} values for Briciclib and ON 013100

Cell line	Briciclib	ON 013100
AGS	10.2 ± 0.4	6.8 ± 0.6
FLO-1	12.2 ± 0.3	10.1 ± 0.5
JEKO-1	9.7 ± 0.7	4.8 ± 0.2
MINO	9.8 ± 0.05	6.7 ± 0.1
MCF-7	15.1 ± 0.8	7.9 ± 0.3
MDA MB 231	13.5 ± 1.2	10.8 ± 0.7

Concentrations in nM

Figure 4. Briciclib or ON 013100 treatment inhibits expression of downstream targets of eIF4E and induces apoptosis:

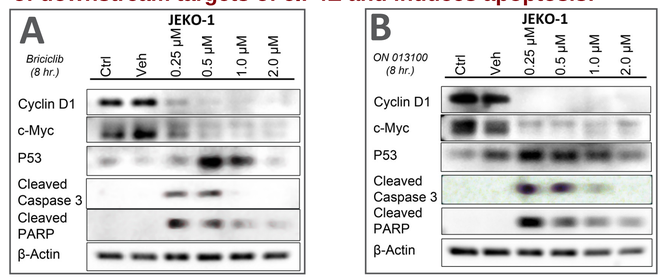


Fig. 4: (A & B) JEKO-1 MCL cancer cells were treated with increasing concentrations of Briciclib or ON 013100 for 8 hrs. and western blot analysis was done to determine protein expression. The data indicates significant inhibition of eIF4E downstream targets (Cyclin D1 and c-Myc) and apoptotic marker proteins (P53, Cleaved Caspase 3, and Cleaved PARP) after treatment with Briciclib or ON 013100 in a dose dependent manner.

CONCLUSION

- Overall our findings indicate that both Briciclib and ON 013100 exhibit similar anticancer activity in various cancer cell lines. Our *in vitro* data emphasize the potential of aforementioned eIF4E- inhibitors in selectively treating hematopoietic and solid cancers.

ACKNOWLEDGEMENTS

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