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The cyclin-dependent kinase 4/6 inhibitor LEE011 (ribociclib) demonstrates antiproliferative effects in neuroendocrine tumor cells *in vitro*

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Aim

To investigate the effects of LEE011 on neuroendocrine tumor (NET) cell growth and signaling *in vitro*

Introduction

Cyclin-dependent kinases (CDKs) are crucial for the cell cycle regulation and alterations of the cell cycle and its regulators are often observed in human malignancies. CDK4/6 in particular orchestrates the G1 phase progression and the G1/S transition

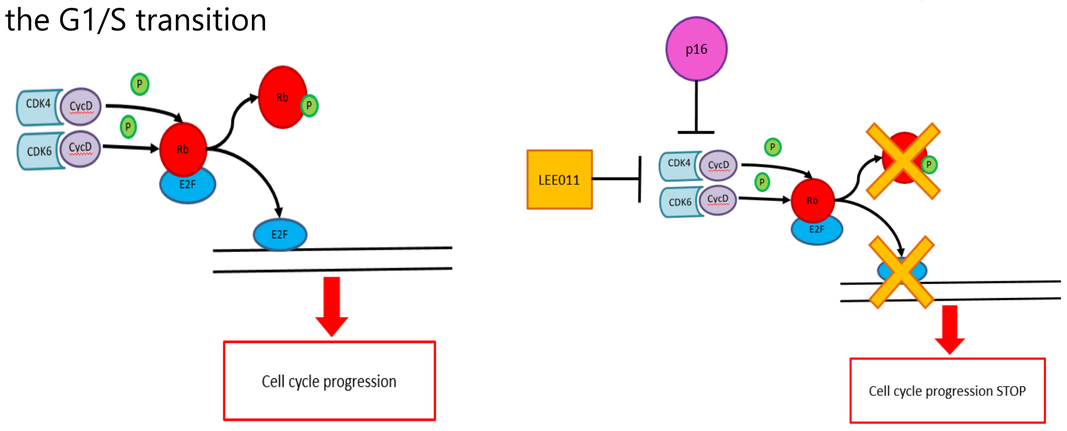

Fig.1: Proposed mode of action of LEE011 on the p16-CDK4/6-Rb pathway by the American Association for Cancer Research (AACR), 2014

Methods

Human pancreatic **BON1**, pancreatic islet **QGP1**, pulmonary **H727** and ileal **GOT1** NET cells
+
different concentrations of **LEE011** (kindly provided by NOVARTIS, Basel) alone and in combination with **5-fluorouracil (5-FU)** (5µM), **Everolimus** (10nM) and different times of incubation (from 48h to 144h)

- Western blot analysis
- Flowcytometric analysis
- Cell viability assay

Statistical analysis was performed using the Kruskal-Wallis Test and Mann-Whitney Test or the student T-Test of the SPSS statistical package, considering $p<0,05$. At least three independent experiments were executed.

Results

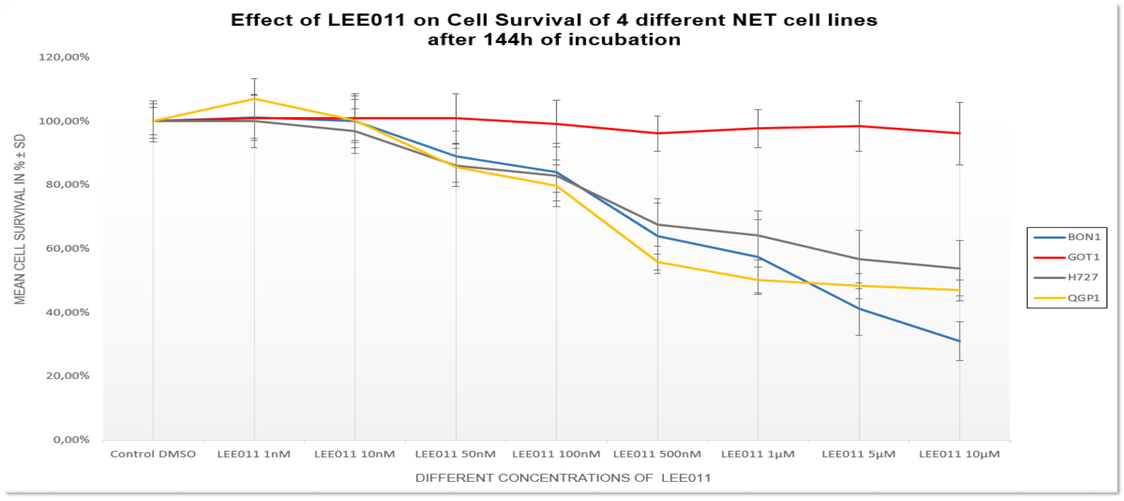

Fig.3: Cell viability of BON1, GOT1, H727 and QGP1 cells after 144h of incubation with different concentrations of LEE011. The most sensitive cell line towards treatment at the highest LEE011 dose (10µM) was BON1 and the least sensitive GOT1 : BON1>QGP1>H727>GOT1

50% inhibitory concentration (IC50) of LEE011 in 3 different NET cell lines after 144h of incubation		
BON1	QGP1	H727
IC50= 2,6 µM	IC50= 1,2 µM	IC50= 10,9 µM

Fig.2: IC50 values of BON1, H727 and QGP1 cells after 144h of incubation with LEE011. The most sensitive cell line towards treatment considering IC50 was QGP1 followed by BON1 and H727: QGP1>BON1>H727

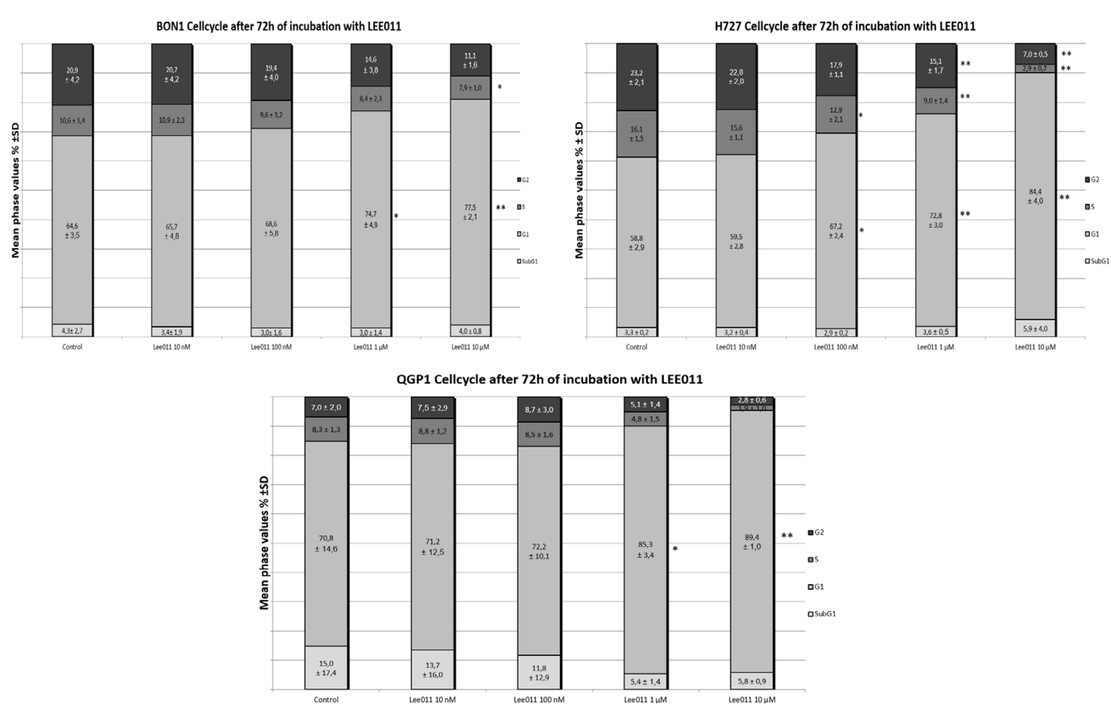

Fig.4: Cell cycle analysis with different concentrations of LEE011 (from 10nM to 10µM) in BON1, H727 and QGP1 cells at an incubation time of 72h. In all three cell lines a dose-dependent G1 arrest was detected, suggesting a cell cycle STOP as a consequence to LEE011 treatment.

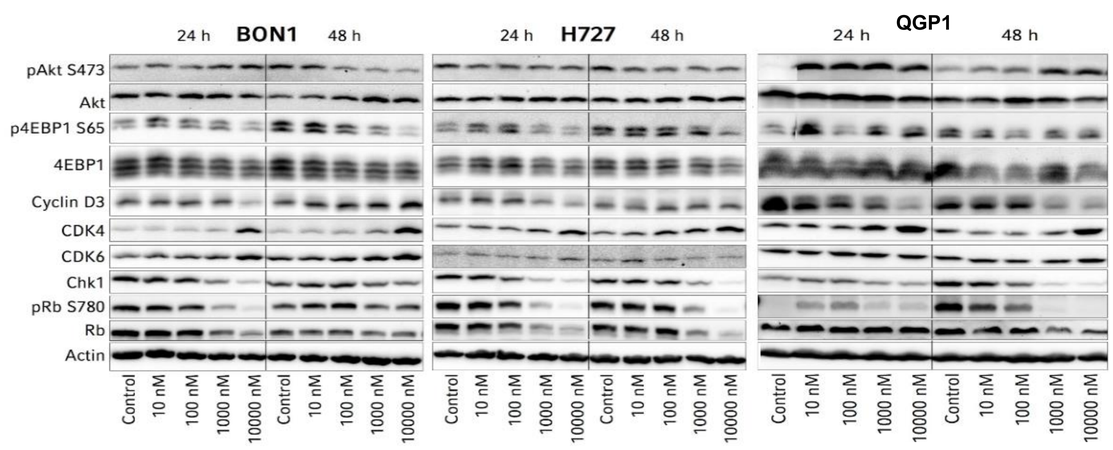

Fig.5: LEE011 showed PI3K-Akt-mTOR pathway suppression, due to a phosphorylation decrease of Akt and the mTOR target 4E binding protein 1after 48h of incubation. As a proof of action mode of LEE011, pRb is downregulated in all three cell lines after 24h and 48h of incubation . A compensatory downregulation of Chk1 is also observed in all three cell lines. Furthermore, a compensatory upregulation of CDK4/6 in response to inhibition was observed in BON1, H727 and QGP1 cells.

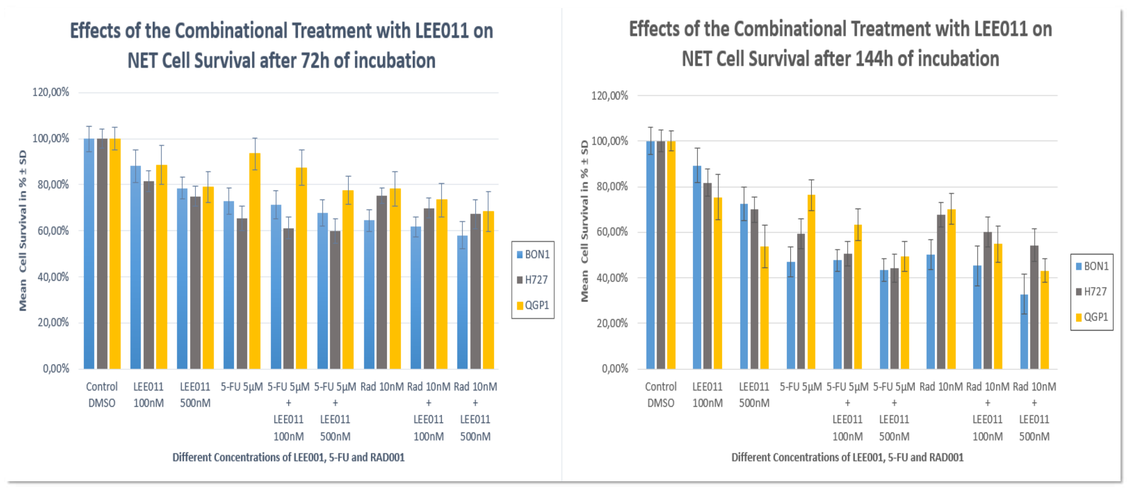

Fig.6: Cell viability of LEE011 alone and in combination with 5-FU and Everolimus in BON1, QGP1 and H727 cells at incubation times of 72h and 144h. In general the combinational treatment strategies showed a significant enhancement in inhibition of cell viability when compared to the respective single treatments in BON1, QGP1 and H727 cells.

Conclusion

Consequently, the CDK4/6 inhibitor LEE011 demonstrates promising anti-cancer properties for NETs *in vitro*. Further pre-clinical studies exploring the putative role of LEE011 in combinational treatments in these cell lines are currently ongoing.

In cooperation with:

FAZIT-STIFTUNG