

Synergistic anti-tumour effects of 13-cis retinoic acid and lovastatin in pancreatic neuroendocrine tumour (BON1) cells through enhanced EGFR inhibition

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Introduction

In our previous studies we found that the combination of 13-cis retinoic acid (13cRA) and lovastatin significantly reduced tumour growth in a mouse pheochromocytoma allograft model, with the lowest microvessel density in the combination-treated tumours Nölting et al., Endocrinology, 2014 Jul). We have now investigated the effect of 13cRA plus lovastatin on neuroendocrine (BON1, H727) and non-endocrine tumour (HepG2, Huh7) cell viability and signalling pathways (EGFR, AKT, ERK, p70S6K) to elucidate the underlying mechanism of action.

Fig.1

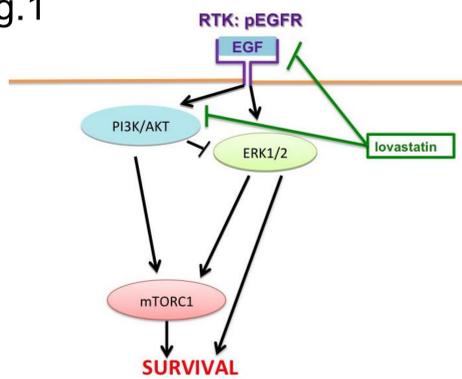


Fig.1: Effects of lovastatin on neuroendocrine tumour cells (modified from Nölting et al, PLOSone, 2015, Dec)

Methods

Cell viability was assessed with the MTS assay, the effect on signalling pathways by Western blotting. For cell viability data, the multiple-comparison Kruskal-Wallis-Test was used, followed by pairwise comparisons with the Mann-Whitney-Test. Interaction effects were analysed with Linear-Mixed-Effects-Models. Statistical significance was defined at $p \leq 0.05$.

Results

Fig.2

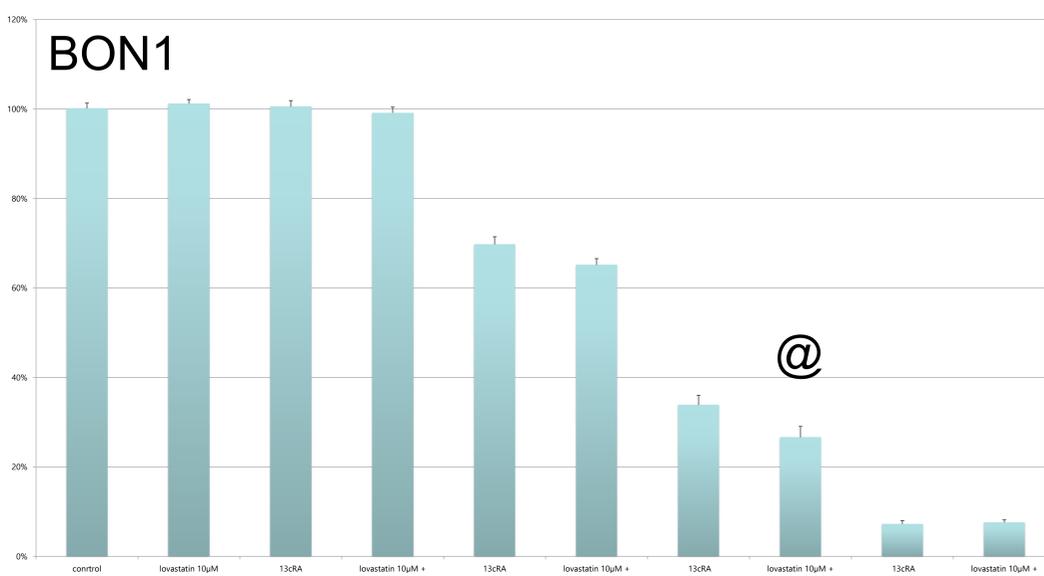


Fig.2: The combination of 10µM lovastatin plus 7.5µM 13cRA shows synergistic inhibition of BON1 cell viability ($p \leq 0.05$) (@)

Conclusion

- Synergistic effect of 13cRA plus lovastatin in BON1 cells at clinically relevant doses, associated with enhanced EGFR inhibition
- Consistent with our *in vivo* data in a pheochromocytoma allograft model showing slowest tumour growth and lowest microvessel density after 13cRA/lovastatin-treatment
- Potentially novel effective drug combination

Fig.3

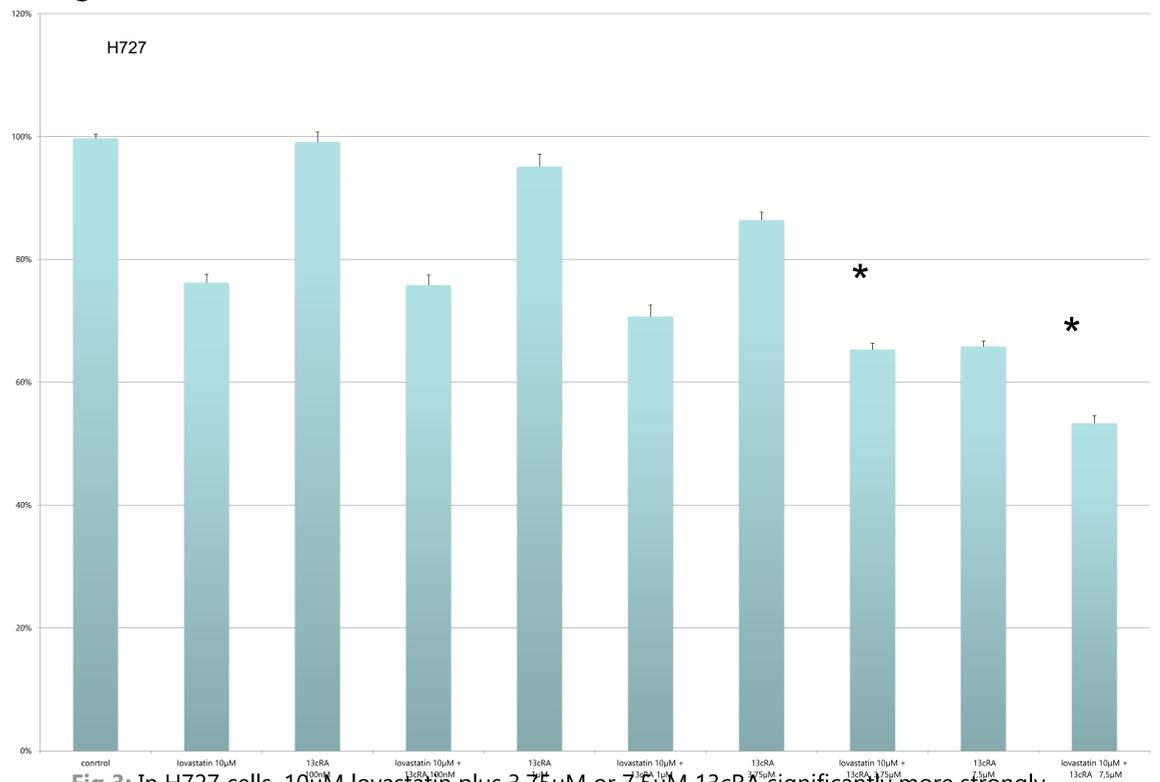


Fig.3: In H727 cells, 10µM lovastatin plus 3.75µM or 7.5µM 13cRA significantly more strongly reduces cell viability than each drug separately ($p \leq 0.05$) (*), but this effect is not synergistic.

Fig.4

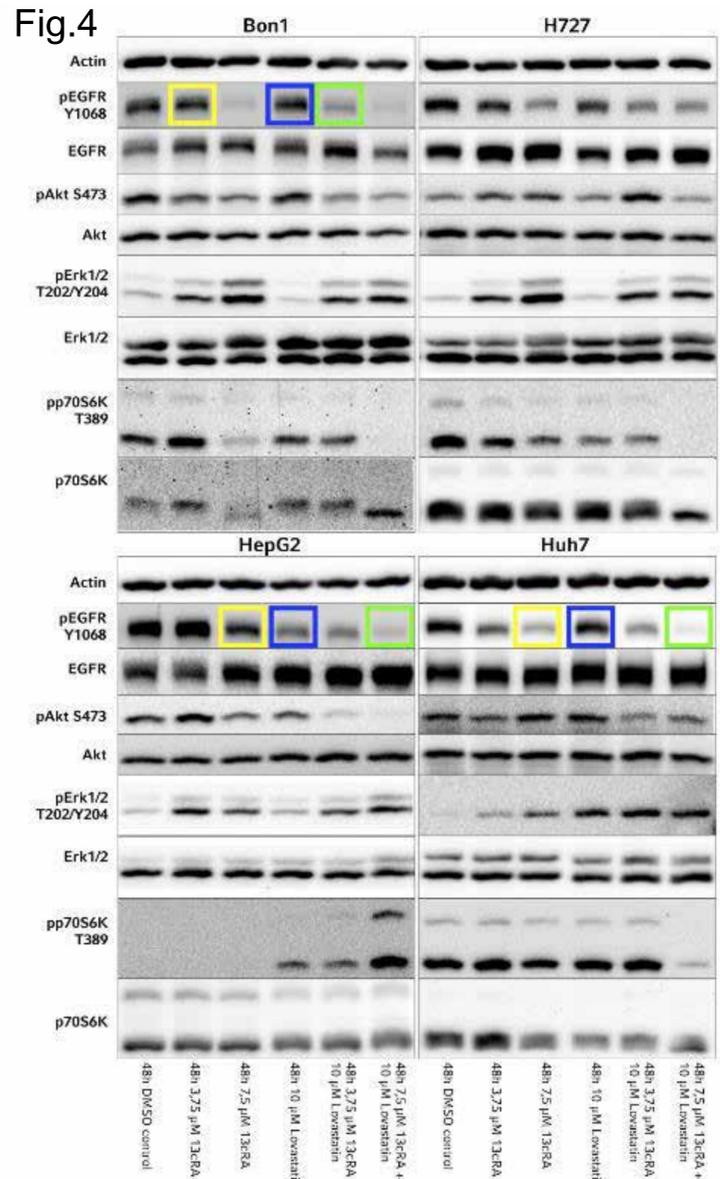


Fig.4: Exemplary western blots of each cell line tested: In BON1, HepG2 and Huh7 cells, combination-treatment (green) decreases pEGFR by more than 50% relative to each drug separately (yellow/blue), and by more than 80% relative to the untreated control. This effect is less pronounced in H727 cells.