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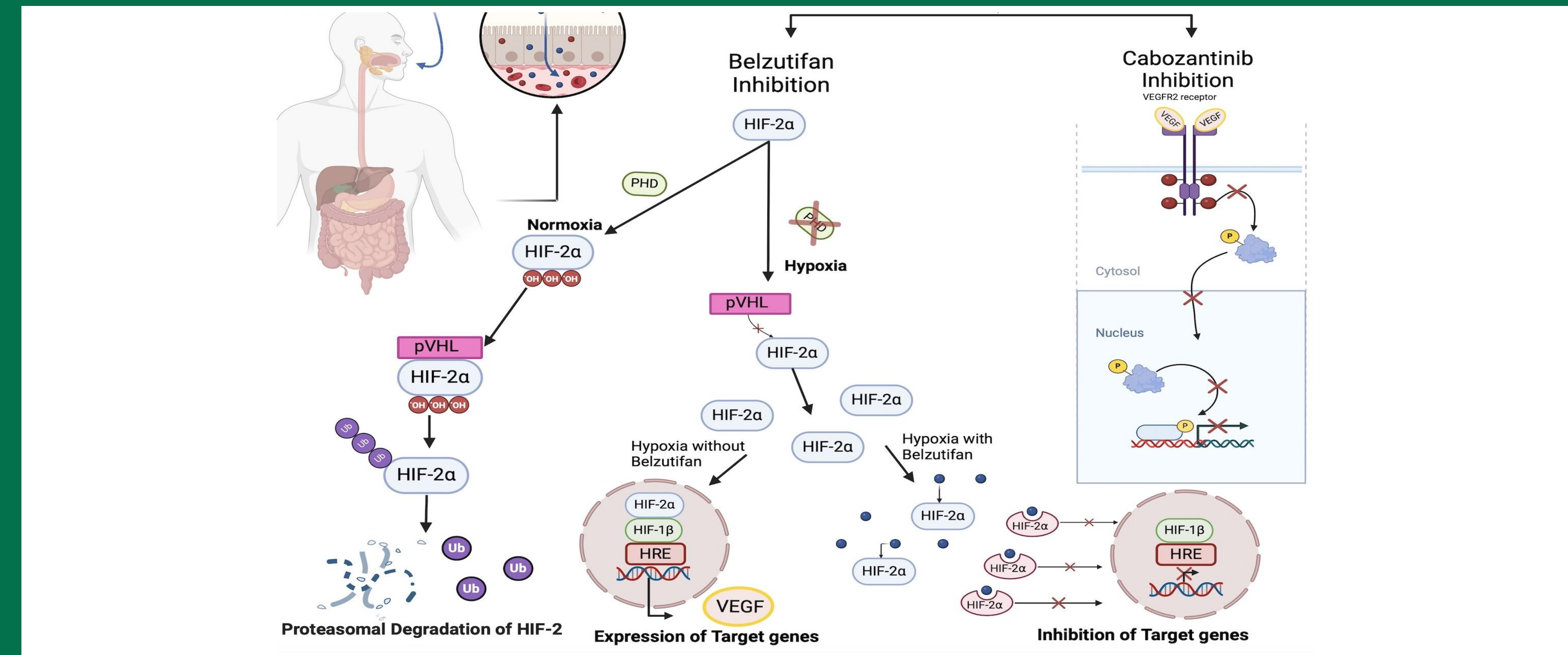
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INTRODUCTION

- Well-differentiated neuroendocrine tumors (NETs) are highly vascularized malignancies with vascular endothelial growth factor (VEGF) pathway playing a key role in regulating angiogenesis.
- Inhibition of angiogenesis has revealed therapeutic efficacy in NETs, most recently with Cabozantinib, a multi-kinase inhibitor targeting VEGF receptor (VEGFR), c-MET, AXL, and RET.
- Belzutifan is a HIF-2 α inhibitor approved for VHL associated tumors. It demonstrated significant antitumor activity, with a 91% objective response rate in patients with pancreatic NETs (pNETs).
- The HIF pathway and angiogenesis are closely interlinked. Combination of angiogenesis and HIF-2 α could repress VEGF at both the transcription and receptor level and overcome resistance.

OBJECTIVES

- Evaluate the synergistic potential of combining Belzutifan and Cabozantinib in NET cell lines.
- Assess the effects of this drug combination under both normoxic and hypoxic conditions.
- Quantify drug synergy using the Zero Interaction Potency (ZIP) synergy score in multiple NET cell lines.
- Measure changes in cell viability with combination treatment using MTT and trypan blue exclusion assays.
- Examine the effects of combined treatment on three-dimensional tumor models using spheroid experiments.
- Investigate the impact of combination therapy on HIF-2 α expression levels through Western blot analysis.



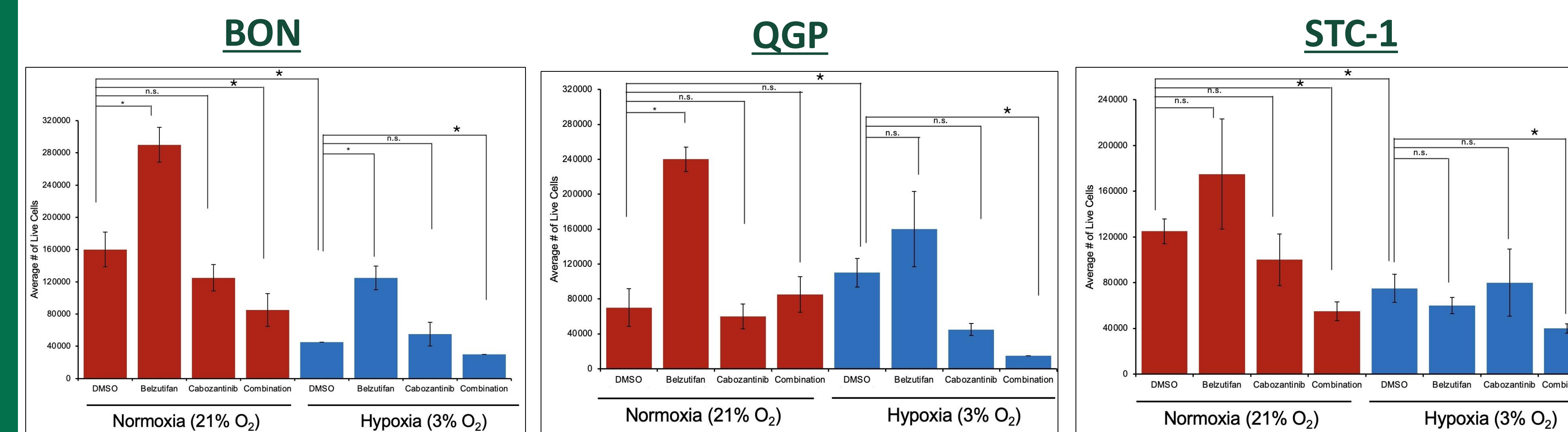
RESULTS

Synergy increases when cells are in Hypoxia

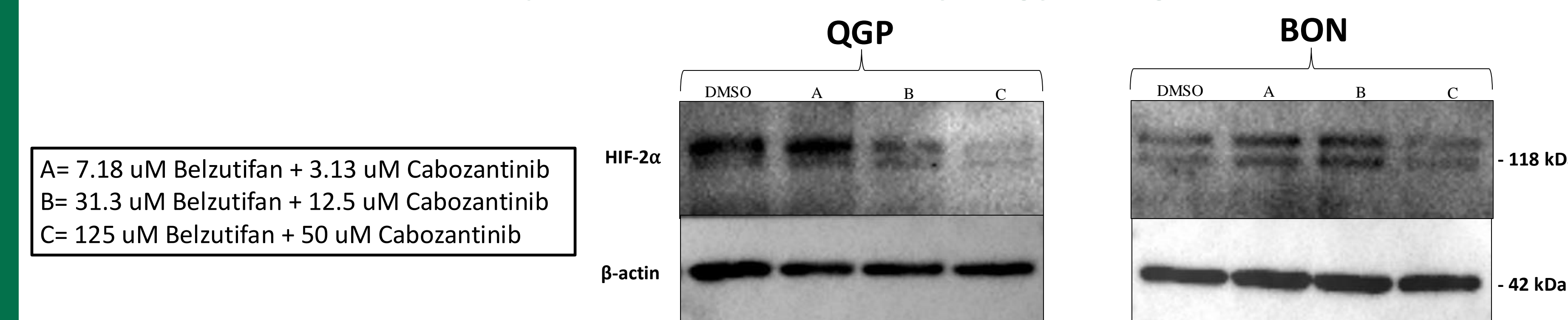
- Score quantifies the synergistic or antagonistic effects of drug combinations by comparing the change in potency of dose-response curves between individual drugs and their combinations.
- Positive scores indicate synergistic effects, and negative scores indicate antagonistic effects.

ZERO INTERACTION POTENCY (ZIP)		
Cell Line	Normoxia	Hypoxia
BON	27.26	34.74
QGP	131.03	168.01
STC-1	0.9	36.89

Synergy dosage decreases cell viability



HIF-2 α expression decreases as synergy dosages increase



METHODS

BON	QGP	STC-1
Established from lymph node metastasis of a human pNET	Established from a primary pNET	Established from intestinal NET in a transgenic mouse model

- Normoxic and Hypoxic conditions simulated with Heracell VIOS 160i incubators. Hypoxia was 3% oxygen.
- Serial dilution drug treatments: Belzutifan (250 μ M to 7.8 μ M) and Cabozantinib (100 μ M to 3.1 μ M)
- Analysis methods: SynergyFinder Plus platform, ZIP synergy score
- Cell viability assays: MTT and trypan blue exclusion
- Western blot analysis for HIF-2 α expression

CONCLUSIONS

- Synergistic effects are observed in non-VHL mutated gastroenteropancreatic (GEP)-NET cells.
- Belzutifan and Cabozantinib have strong synergistic potential as a combination therapy in GEP-NET cell lines.
- Western Blot shows that the target protein, HIF-2 α , is inhibited more strongly by the drug combination in GEP-NET cell lines.

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