

EP02.09: Novel Drug Co-Therapy: Cisplatin and the DNA PKcs Inhibitor KU60648

INTRODUCTION

- Resistance to platinum-based chemotherapy is a major challenge in NSCLC
- DNA-PKcs (DNA-dependent protein kinase catalytic subunit) is a key regulator of non-homologous end joining (NHEJ)-mediated DNA double-strand break repair (DSB).
- MSH3 is a DNA mismatch repair protein that also has a role in coordinating homologous repair (HR)-mediated DSB repair.^{1,2}
- 15-20% of NSCLC cases may harbour some HR repair defects
- Defects in HR and MSH3 increase sensitivity to DNA-PKcs inhibition.³
- KU60648 is a small molecule inhibitor of DNA-PKcs
- KRAS mutations are a driver of NSCLC.
- The KRAS-mutated A549 cells are resistant to KU60648

AIMS & HYPOTHESIS

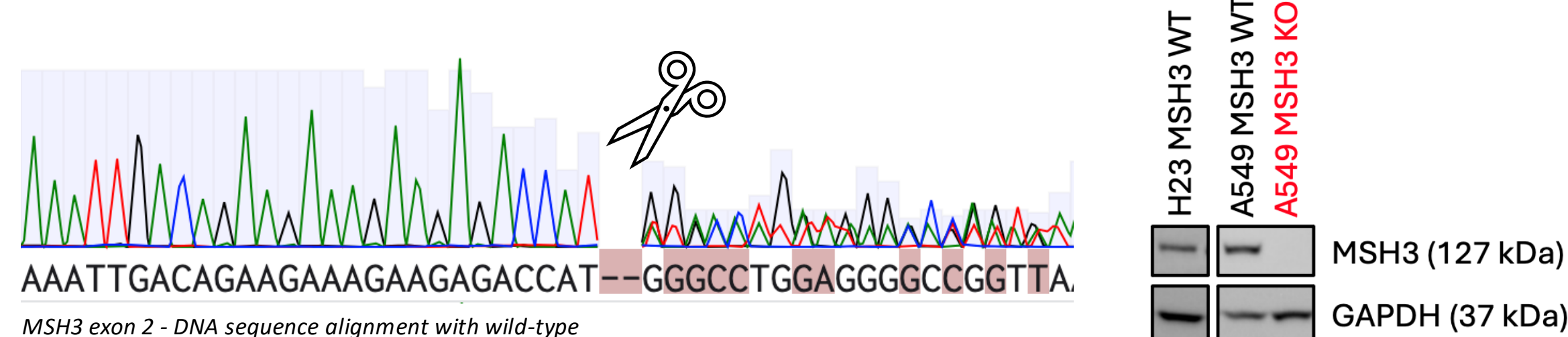
- Investigate if MSH3 deletion will sensitise A549 cells to KU60648 monotherapy or in combination with cisplatin
- Hypothesise that combining KU60648 with cisplatin could exploit DNA repair vulnerabilities to enhance therapeutic response in lung cancer.

METHODS

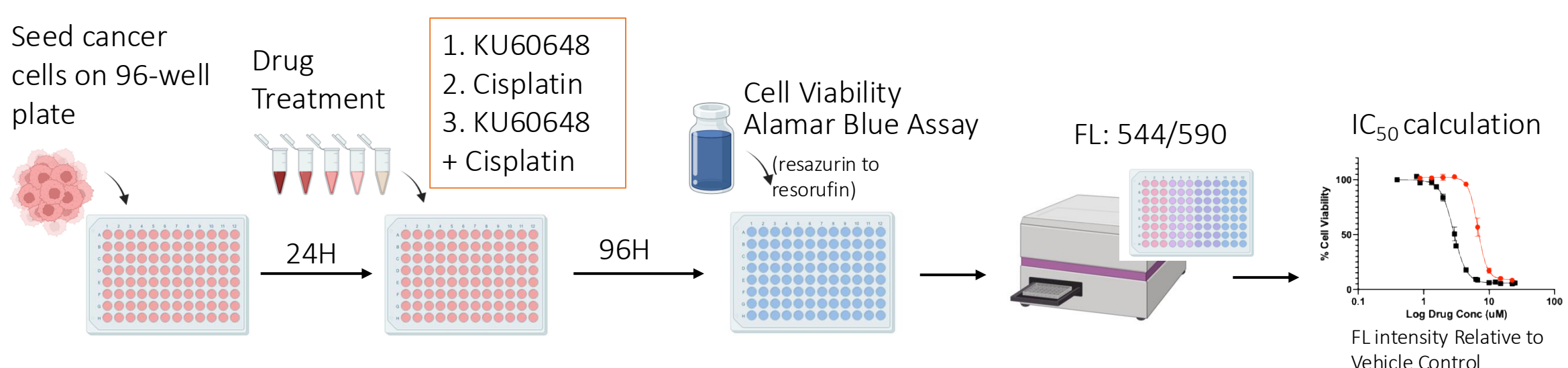
KRAS-mutated lung adenocarcinoma cells lines:

- A549 (G12S) and H23 (G12C)

Create CRISPR-Cas9 knockout A549 cells:



Treat cells with KU60648 + Cisplatin:



Drug synergy analysis: Bliss combination index (CI).^{4,5}

Bliss CI < 1	Synergy
Bliss CI = 1	Additive effect
Bliss CI > 1	Antagonism

RESULTS

Greatest cell death at 0.5 - 1xIC₅₀ with the drug combination **Minimal difference in dose response with the monotherapies**

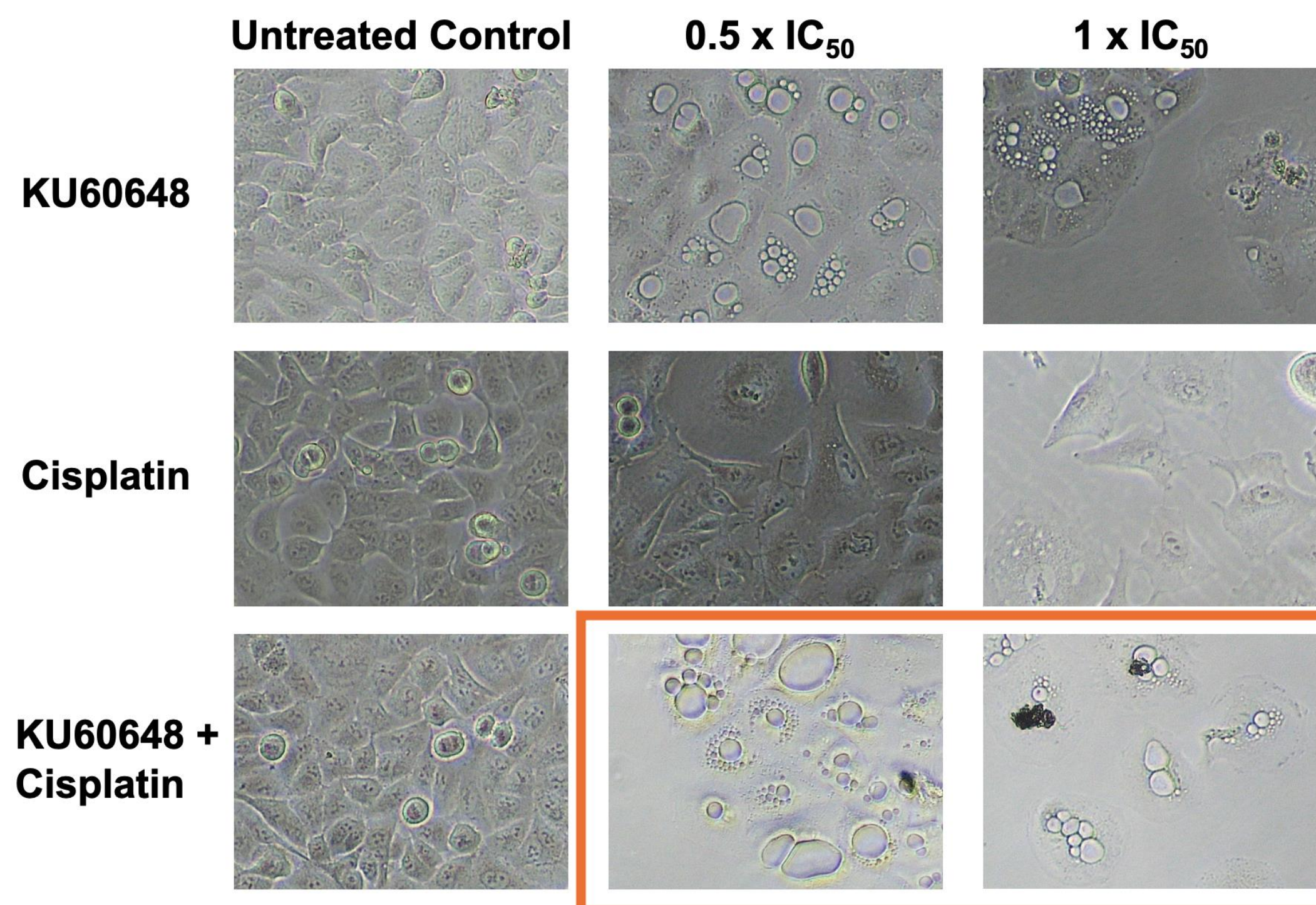


Figure 1: Greater cell death observed at the combined drug doses of KU60648 + cisplatin at both 0.5 X IC₅₀ and 1 X IC₅₀ (orange box) compared to the individual monotherapies and untreated control. Representative image of H23 cells taken at 40X magnification on a Nikon Eclipse Ts2 inverted microscope 96H post-treatment and prior to the addition of Alamar Blue.

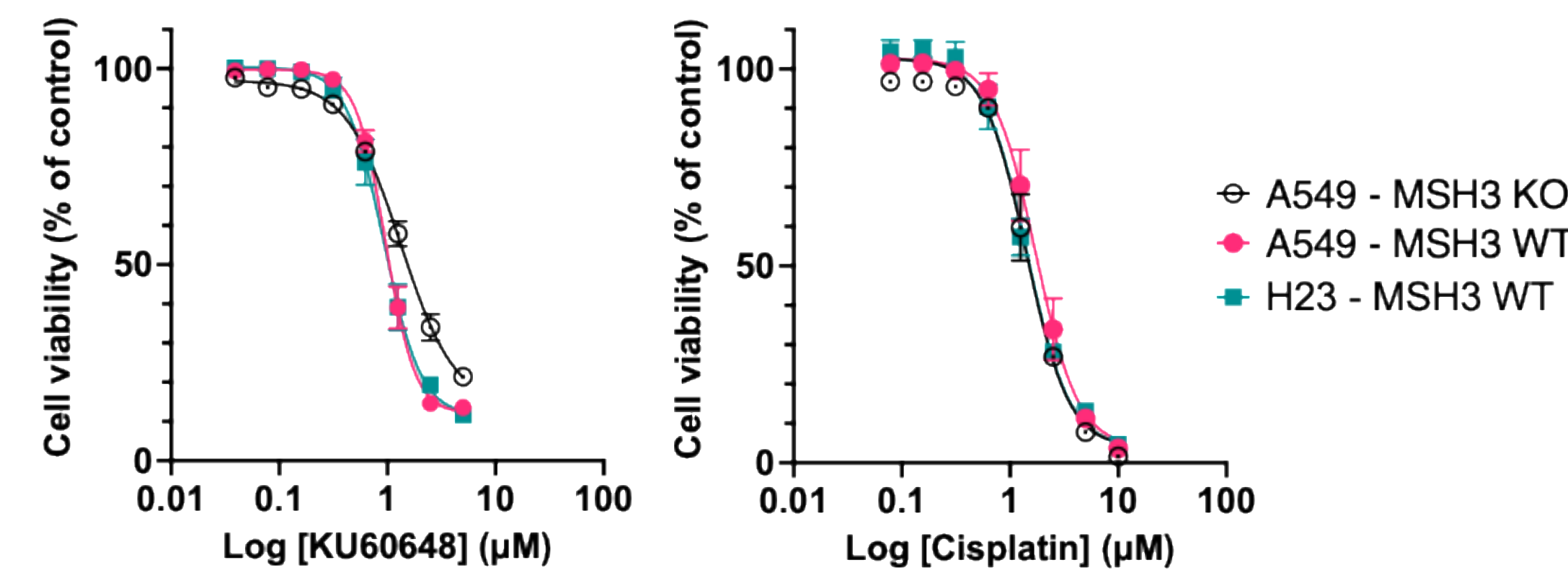


Figure 2: Dose response curves of KU60648 and Cisplatin showing a similar response to KU60648 in both MSH3 WT cells and a slight resistance in the MSH3 KO cells. No difference seen in cisplatin response across all 3 cell lines. Dose-response curves generated in GraphPad Prism, [inhibitor] vs. response - variable slope (four-parameter). All data presented as mean ± SEM (n=3-5).

Table 1: Average IC₅₀ of KU60648 and cisplatin in lung cancer cells

Cell Line	Average IC ₅₀ of KU60648 (μM)*	Average IC ₅₀ of Cisplatin (μM)
A549 - MSH3 KO	1.33 ± 0.19	1.55 ± 0.33
A549 - MSH3 WT	0.96 ± 0.07	1.78 ± 0.56
H23 - MSH3 WT	0.92 ± 0.19	1.39 ± 0.18

* IC₅₀ values determined using non-linear regression analysis in GraphPad Prism from the dose-response curves. Data are expressed as mean ± SD (n=3-5).

Synergy between KU60648 and cisplatin in all cell lines with the greatest degree of synergy in A549-WT cells

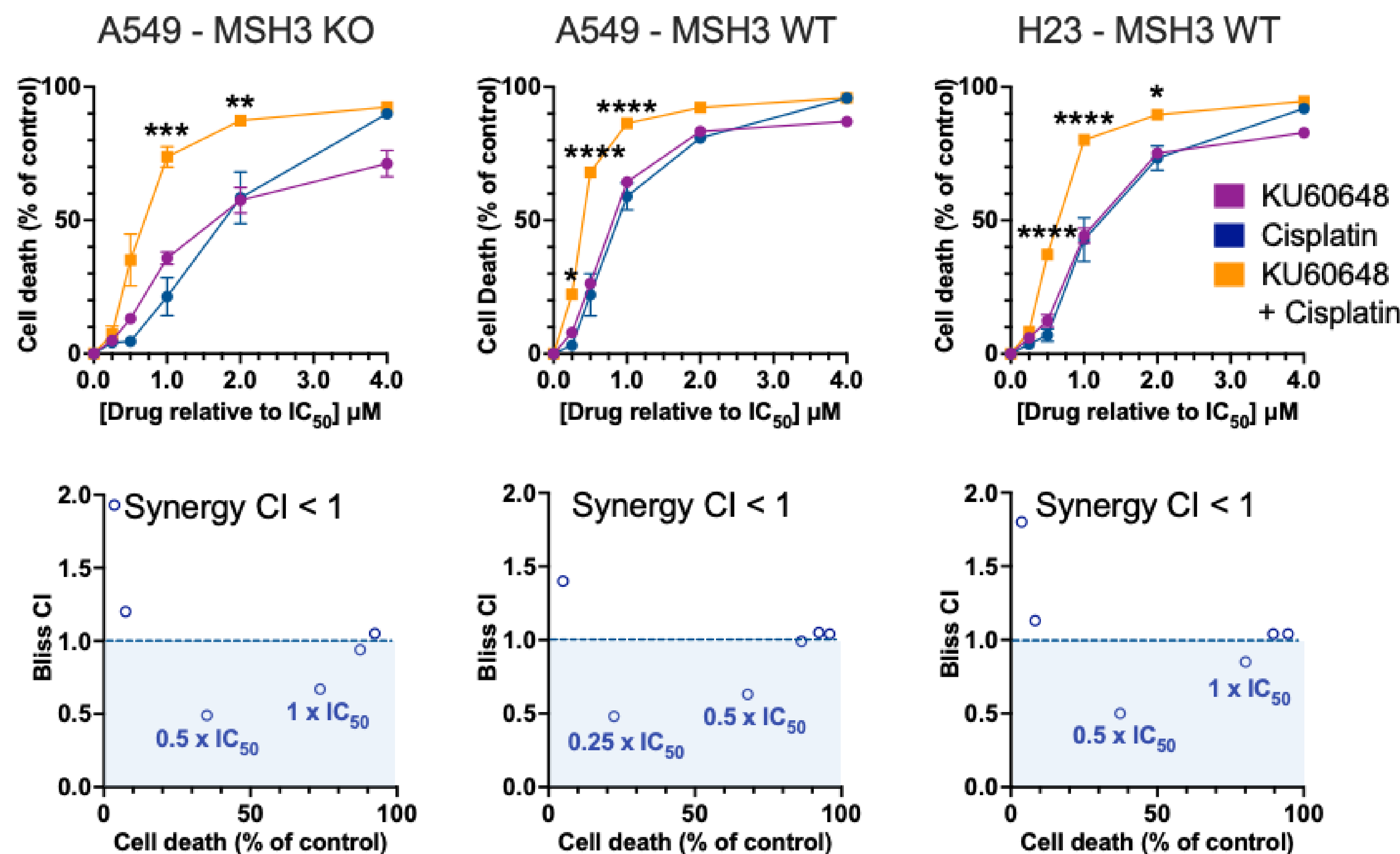


Figure 3: Synergistic effects of KU60648 and cisplatin were observed in all three cell lines, with the greatest degree of synergy observed in MSH3 WT compared with MSH3 KO cells. Cells were treated with increasing concentrations of KU60648 (purple) and cisplatin (blue) monotherapies or in combination (orange), as multiples of the IC₅₀, and presented as percentage of cell death. P-values were determined using a one-way ANOVA with a Tukey's post-hoc test. All data were presented as mean ± SEM (n=3). Synergy was determined through the Bliss combination index analysis.

A549-WT cells are more sensitive to the combination treatment

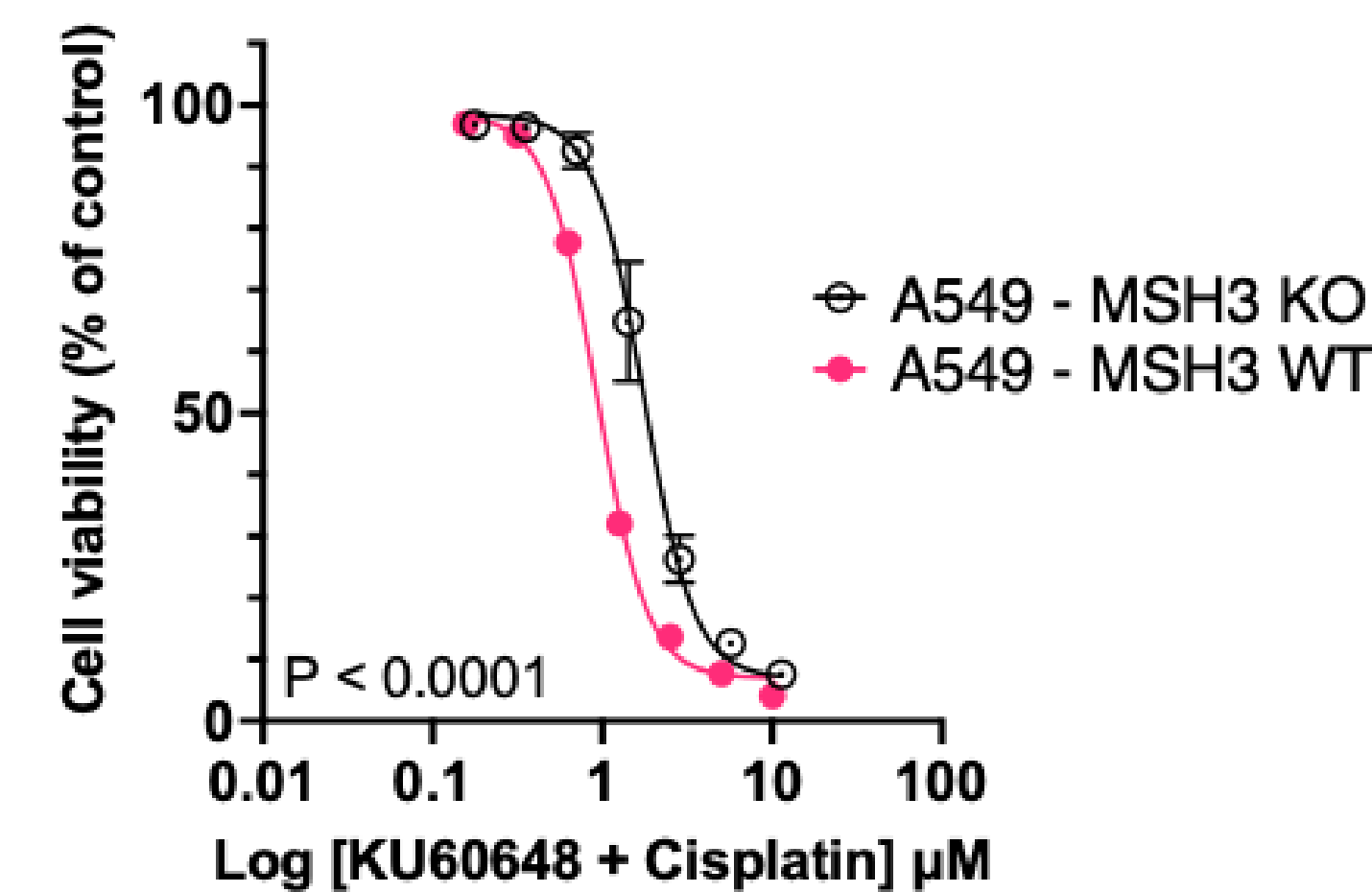


Figure 4: A549 MSH3 WT cells (pink) are the most sensitive to the combination treatment of KU60648 + cisplatin. Dose-response curves generated in GraphPad Prism, [inhibitor] vs. response - variable slope (four-parameter). P-value determined using an extra sum-of-squares F test comparing the IC₅₀ parameter. All data presented as mean ± SEM (n=3).

DISCUSSION AND CONCLUSION

- Synergy between KU60648 and cisplatin in KRAS-mutated cells suggests a promising treatment strategy.
- All three cell lines showed synergy with the combination treatment, but contrary to our hypothesis, MSH3 deletion did not enhance sensitivity to KU60648-cisplatin in A549 cells.
- Instead, slightly reduced synergy was observed in MSH3 KO cells, suggesting that the synergy may partly depend on MSH3-proficient DNA damage recognition and replication-coupled DSB formation.
- Synergy between KU60648 and cisplatin could be further tested in mouse models and ultimately in a clinical trial setting.
- Lower drug dose in combination treatment may lead to a decrease in cisplatin toxicity

REFERENCES & ACKNOWLEDGEMENTS

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Funded by: Woolcock Centre for Lung Cancer Research and the Australian Government Research Training Program PhD scholarship

AI was not used in the development of this poster.