

Preclinical Testing of Metabolic Inhibitors with Erlotinib in Renal Medullary Carcinoma

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Introduction

- Renal Medullary Carcinoma is an aggressive form of kidney cancer that predominately affects **young Black individuals** with **sickle cell trait**.¹
- Increased hypoxia, including exercise-induced hypoxia, in combination with **sickle cell trait** is a **substantial risk factor for RMC**.¹
- RMC is often diagnosed at stage IV and has a poor prognosis with a 13-month survival rate. It is **often refractory** to its first line of **treatment** (platinum-based chemotherapy).^{1,2}

Background

- PDX models** of RMC show successful tumor growth inhibition with Erlotinib, an epithelial growth factor signaling inhibitor.
- Metabolism has been found to be significantly different in RMC tissue compared to normal kidney tissue.³
- In addition, EGFR signaling has been found to induce metabolic changes in cells.⁴

Goals / Hypothesis

- Goal:** Assess and determine the optimal combination of metabolic inhibitor with Erlotinib to reduce RMC cell proliferation.
- Hypothesis:** Our hypothesis is that addition of a metabolic inhibitor will increase the efficacy of Erlotinib in treating RMC.

Methods

- Using patient-derived UOK360⁵ cells, three drug assays on black 96-well plates were completed. CellTiter-Glo Assay was then used to quantify the number of viable cells following each study.
- The three drug assays were two monotherapy assays and one combined therapy assay.

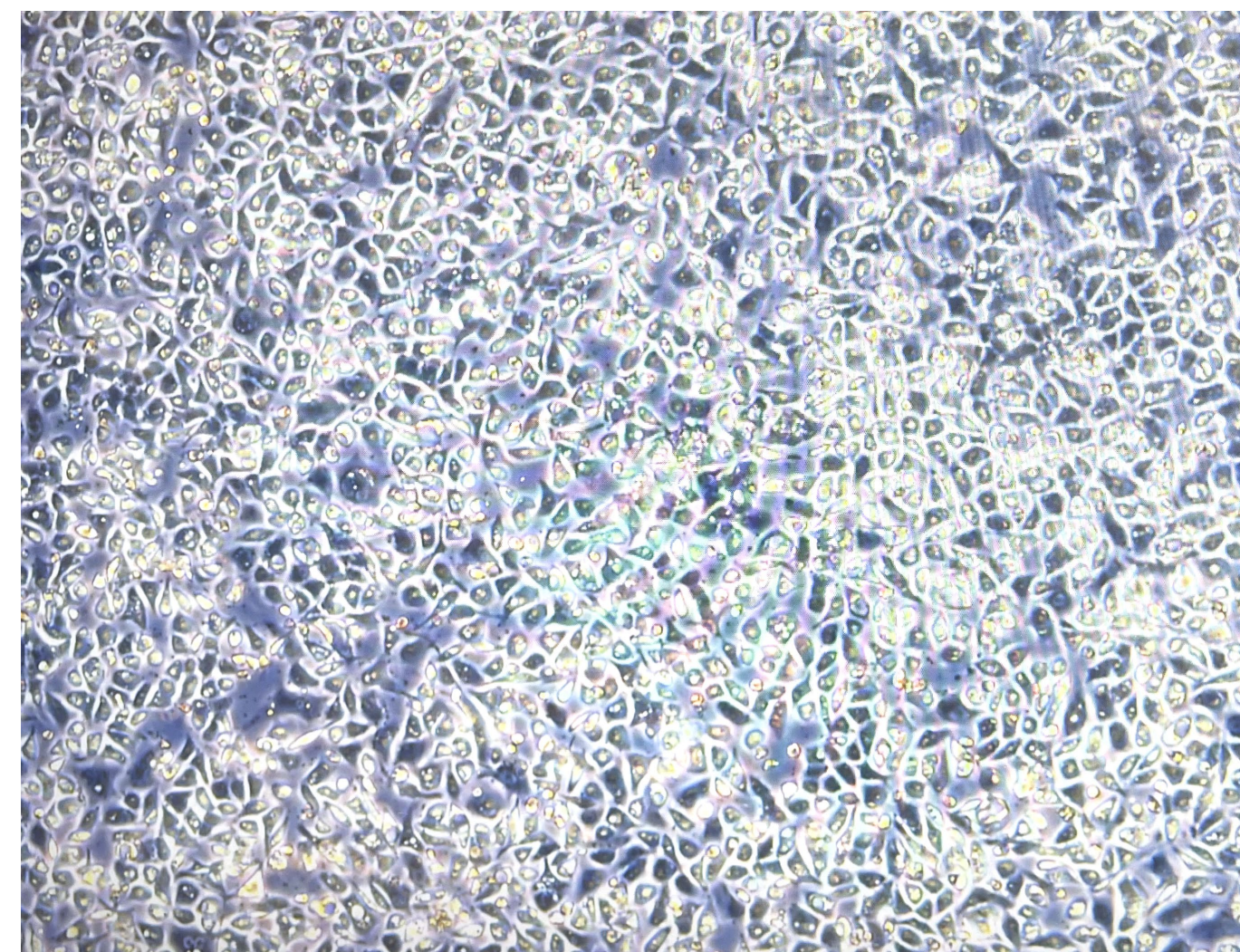


Figure 1. UOK360 cell line derived from tumor sample of 41-year-old-female with sickle cell trait and RMC.⁵

Results

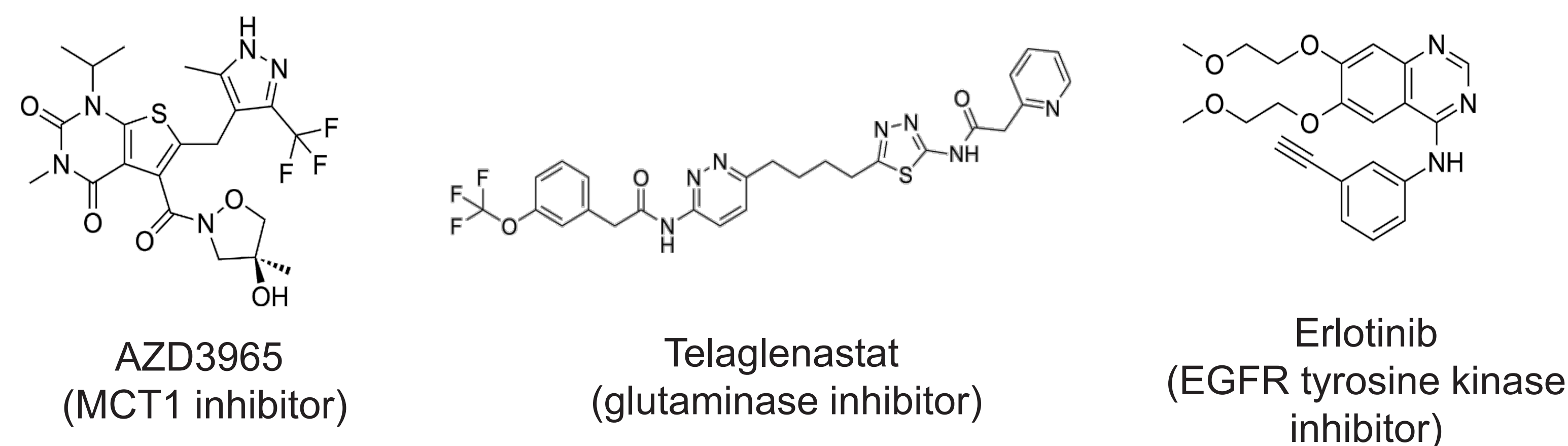


Figure 2. Molecular structures of metabolic inhibitors.

Monotherapy

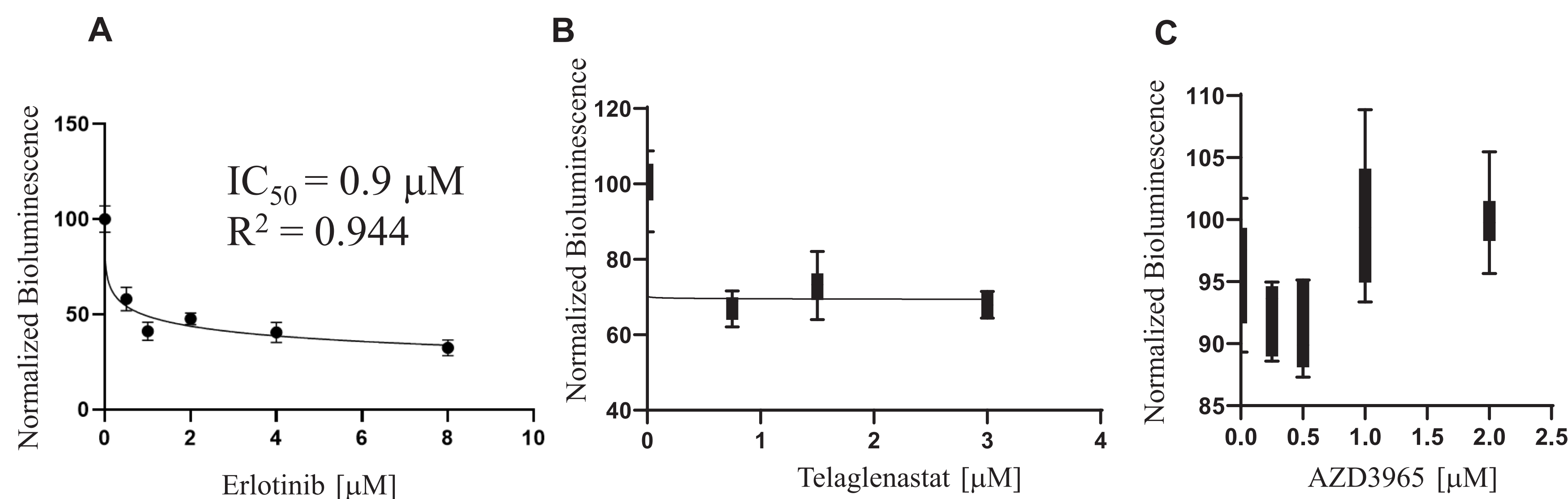


Figure 3. Effect of various concentrations of Erlotinib, Telaglenastat, and AZD3965 on RMC cell proliferation, measured by amount of bioluminescence. 7,000 cells per well were plated in each 96-well plate. Following a 24-hour incubation period, three 96-well plates were used to test monotherapy efficacies at various concentrations. A vehicle was also tested (DMSO), which we used to normalize the graph to a common scale of 100%. CellTiter-Glo was used to quantify ATP and indicate number of metabolically active cells following 48-hour drug assay. [3A.] RMC cells treated with Erlotinib (EGFR inhibitor). See decreasing levels of bioluminescence with increasing concentration of drug as expected. Lowest bioluminescence seen at 8 μM (highest concentration). [3B.] RMC cells treated with Telaglenastat. See significant drop in bioluminescence at 750 nM but does not drop further at higher concentrations. [3C]. RMC cells treated with AZD3965 (Monocarboxylate transporter, MCT1, inhibitor). No significant activity seen. Bioluminescence similar to 0 μM concentration reading for all concentrations of drug.

Dual Therapy

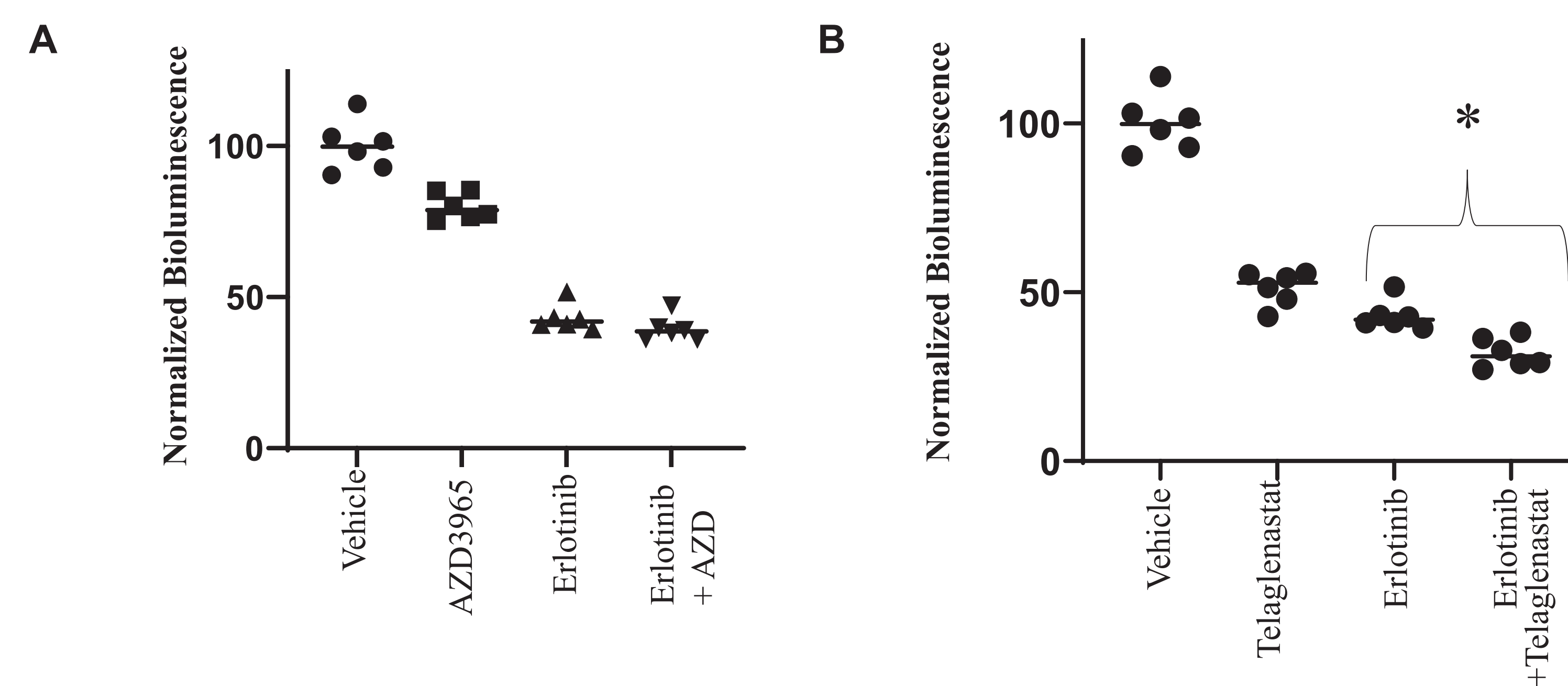


Figure 4. Effect of 48-hour treatment of 500 nM of Erlotinib alone and combined with 500 nM AZD3965 and 750 nM of Telaglenastat. [4A.] Little reduction is observed with AZD3965 alone (500 nM) and we observe no increase in Erlotinib's ability to inhibit cell proliferation with dual therapy (Erlotinib + AZD3965). [4B.] We observe reduction in cell proliferation when cells are treated with Telaglenastat (750 nM) or Erlotinib alone (500 nM). In addition, we observe an additive effect with dual therapy (Erlotinib + Telaglenastat) compared to Erlotinib alone (* P value < 0.02).

Conclusions

- On UOK360 RMC cells:
- We observed cell proliferation reductions with Telaglenastat and no activity with AZD3965.
 - Calculated an IC₅₀ of 0.9 μM for Erlotinib.
 - We observed a statistically significant cell proliferation reduction when 500 nM Erlotinib is combined with 750 nM Telaglenastat (P < 0.02).

Future Directions

- Test compounds in other RMC cell lines (RMC2C, UOK353).
- Planning on testing other metabolic inhibitors (V-9302).
- Planning on testing compounds in a 7-day incubation study. Cell proliferation will be determined using crystal violet staining.
- Planning on testing efficacious dual therapy combinations in RMC mouse models.

Limitations

- Our work was performed using in vitro cell culture.
- We used only one RMC cell line.
- Used ATP levels to quantify levels of cell proliferation.

References

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