

P2.049: Effect of Combined Inhibition of mTOR and JNK as a Therapeutic Strategy for Thymic Carcinoma

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INTRODUCTION

- Thymic carcinoma is a rare malignancy with a poor prognosis, and treatment options for advanced disease remain limited¹.
- The mechanistic target of rapamycin (mTOR), a serine/threonine kinase downstream of PI3K/AKT signaling, regulates cell proliferation and survival and has been pursued as a therapeutic target across multiple malignancies. Activation of this pathway has also been reported in thymic carcinoma².
- The mTOR inhibitor everolimus was evaluated in thymic carcinoma; however, its single-agent activity was limited, with a median progression-free survival of 5.6 months².
- The mechanisms of resistance to everolimus remain unclear, underscoring the need for combination strategies to overcome it.

METHODS

Cell lines and reagents

Four human thymic carcinoma cell lines (MP57, ThyL-6, Ty-82, and T1889) were used. Everolimus (mTOR inhibitor) and SP600125 (JNK inhibitor) were used.

Statistical analysis

Comparisons among groups were performed using one-way ANOVA with Tukey's multiple comparisons test (GraphPad Prism). $P < 0.05$ was considered statistically significant.

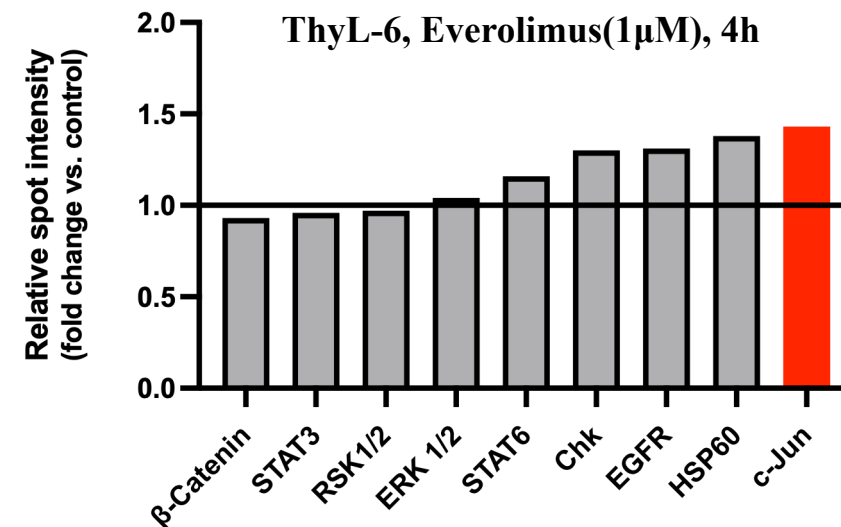
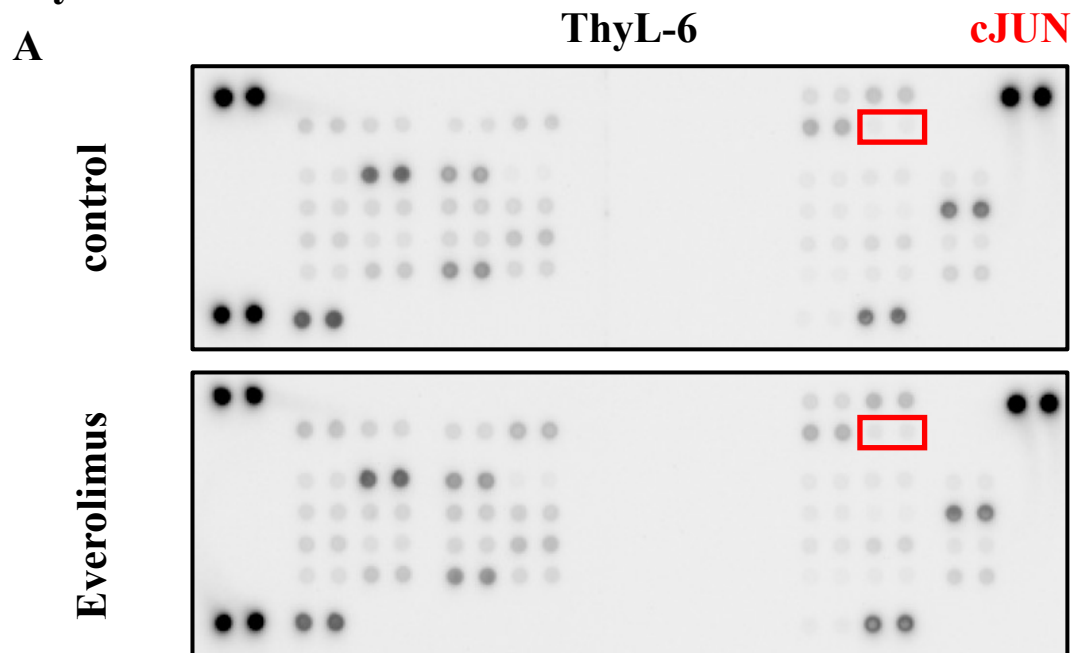
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, not significant.



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RESULTS

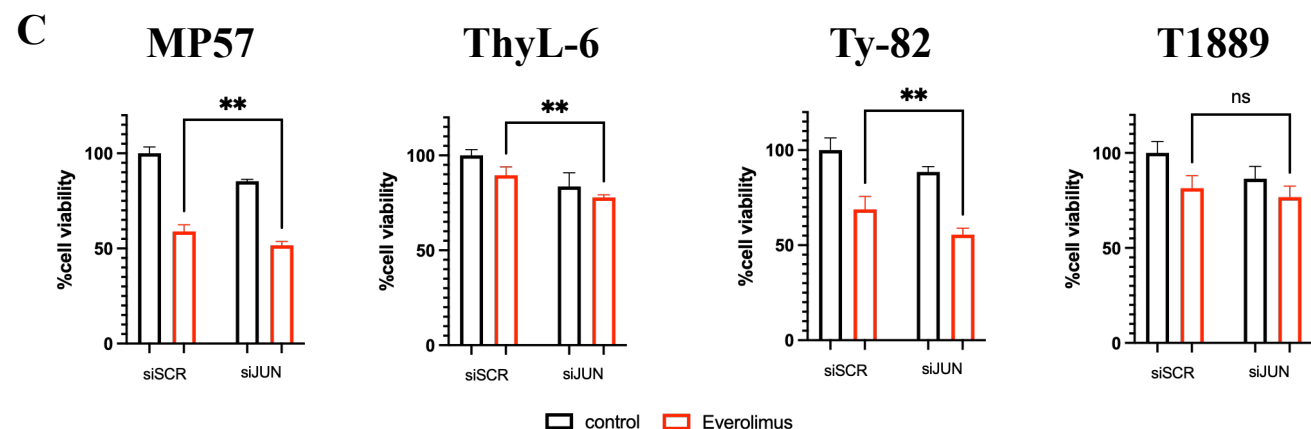
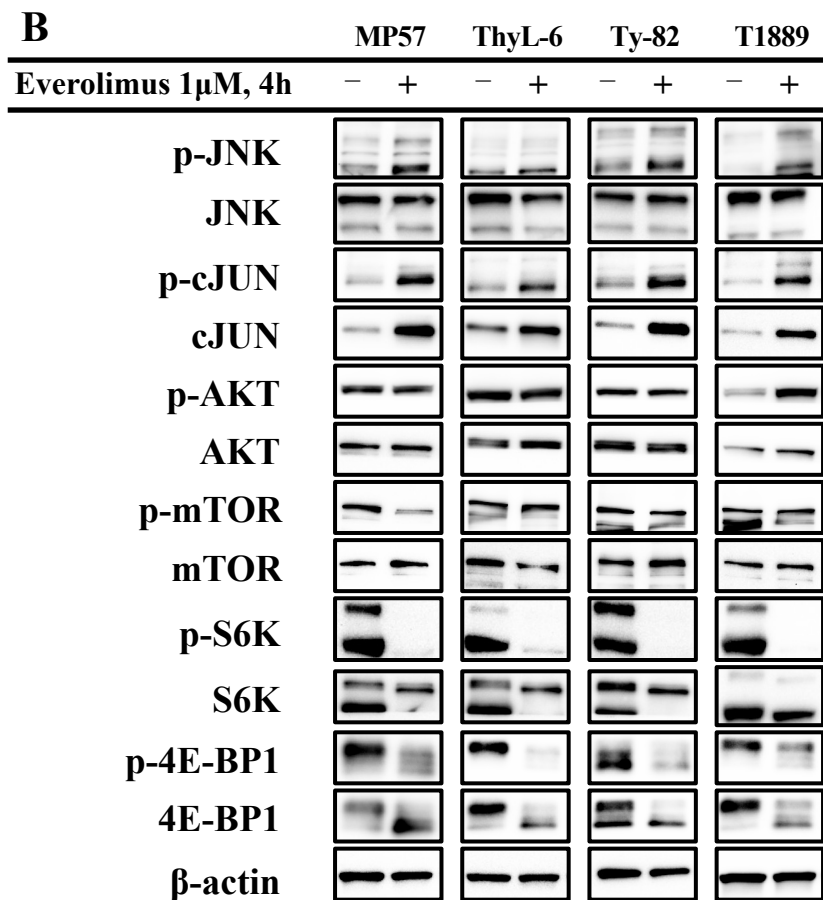
Phospho-kinase array screening identified c-Jun as the most strongly induced protein following everolimus exposure in thymic carcinoma cells.



A Phospho-kinase array analysis of ThyL-6 cells treated with everolimus (1 μ M) or control for 4 h. Left, representative array images. Right, spot densities were background-subtracted, normalized to the internal reference spots, and expressed as fold change relative to control; the 9 targets with the greatest change are shown. c-Jun (red) showed the highest induction.

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Everolimus suppressed mTOR signaling and induced phosphorylated and total c-Jun in all four cell lines. However, JUN knockdown enhanced its antiproliferative effect only in MP57, ThyL-6, and Ty-82, indicating that compensatory c-Jun activation limits mTOR inhibition in these lines.



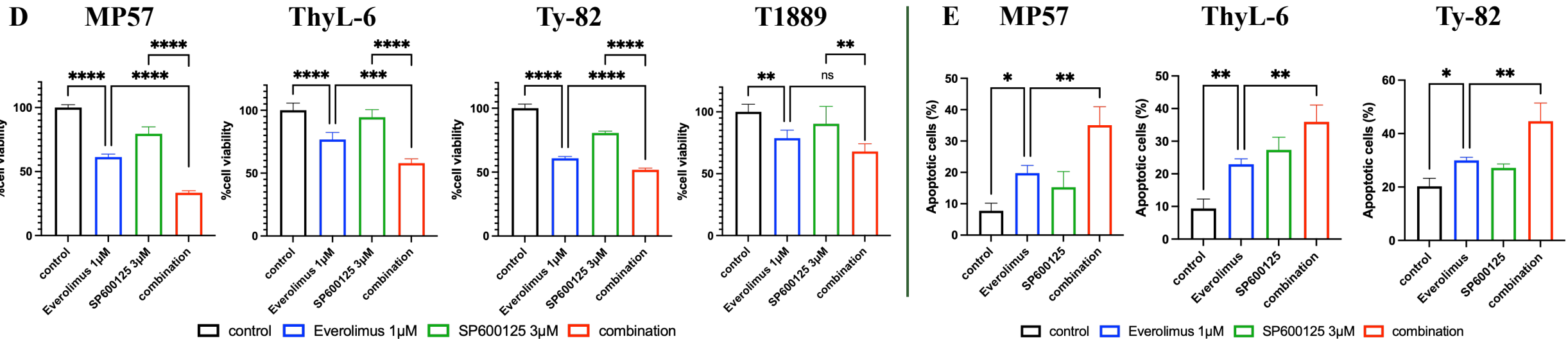
B Western blotting of MP57, ThyL-6, Ty-82, and T1889 cells incubated with medium only or everolimus (1 μM) for 4 h.

C MP57, ThyL-6, Ty-82, and T1889 cells transfected with control (siSCR) or JUN-specific (siJUN) siRNA were incubated with medium only or everolimus (1 μM) for 72 h, beginning 24 h after transfection. Cell viability was assessed using MTT assays.



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Pharmacological JNK inhibition with SP600125 reproduced this effect: combined inhibition of mTOR and JNK significantly suppressed cell proliferation, enhanced apoptosis, and attenuated migration.

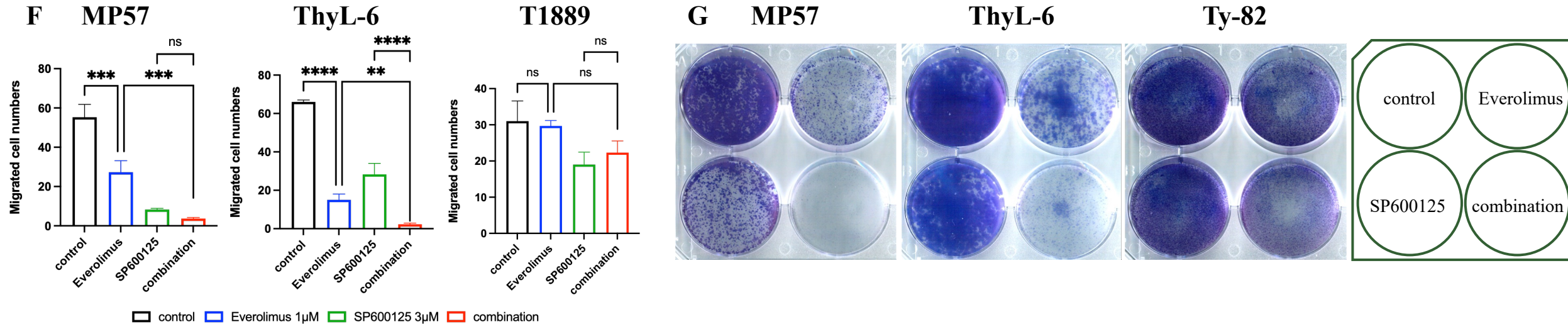


D MP57, ThyL-6, Ty-82, and T1889 cells were incubated with medium only, everolimus (1 μ M), SP600125 (3 μ M), or the combination for 72 h. Cell viability was assessed using MTT assays.

E MP57, ThyL-6, and Ty-82 cells were treated as in (D) for 48 h. Apoptosis was quantified as the proportion of annexin V-positive cells by flow cytometry.

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Pharmacological JNK inhibition with SP600125 reproduced this effect: combined inhibition of mTOR and JNK significantly suppressed cell proliferation, enhanced apoptosis, and attenuated migration.

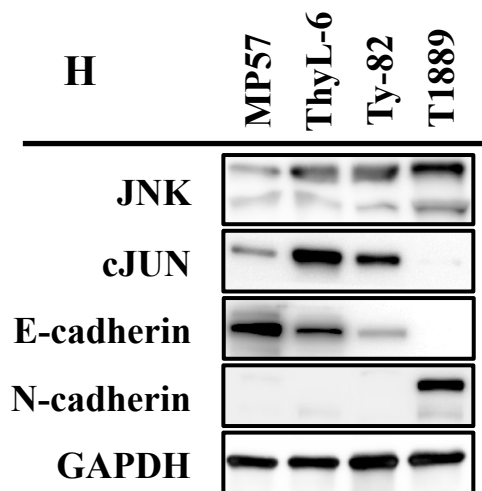


F MP57, ThyL-6, and T1889 cells were treated as in (D). Migration was evaluated using Transwell assays.

G Visualization with crystal violet staining of MP57, ThyL-6, and Ty-82 cells treated with medium only, everolimus (1 µM), SP600125 (3 µM), or the combination for 9 days.

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Cell lines sensitive to the combination displayed an epithelial phenotype, whereas the resistant T1889 line showed a mesenchymal phenotype with high N-cadherin expression.



H Western blotting of untreated MP57, ThyL-6, Ty-82, and T1889 cells.

DISCUSSION AND CONCLUSION

- Activation of JNK/c-Jun represents a mechanism that limits the efficacy of mTOR inhibition in thymic carcinoma.
- Combined inhibition of mTOR and JNK suppressed cell proliferation, enhanced apoptosis, and attenuated migration, supporting this combination as a novel therapeutic strategy for thymic carcinoma.
- c-Jun was induced in all cell lines, yet dependence on it differed by EMT status — suggesting that the epithelial–mesenchymal phenotype, rather than c-Jun induction itself, may predict benefit from this combination. Further evaluation is required to establish EMT status as a predictive biomarker.

REFERENCES & ACKNOWLEDGEMENTS

1) Mediastinum. 2024;8:36.

2) J Clin Oncol. 2018;36(4):342-349.

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Claude (Anthropic) was used to assist with language editing and layout of this poster. All experimental data, analyses, and interpretations were generated and verified by the authors, who take full responsibility for the accuracy and integrity of the content.

