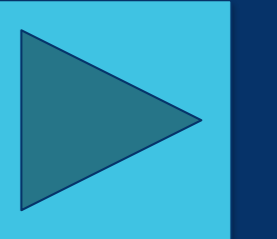


Rational design of a combination therapy for MTC and evaluation of an imaging biomarker for prediction of treatment response

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Introduction

Medullary thyroid carcinoma (MTC) originates from the thyroid gland neuroendocrine C-cells. Treatment options for progressive, metastatic MTC patients consist of the FDA-approved tyrosine kinase inhibitors (TKIs), Vandetanib and Cabozantinib. Another TKI, Nintedanib, is currently undergoing Phase II clinical trials. TKI resistance is common and improving current treatments is needed.

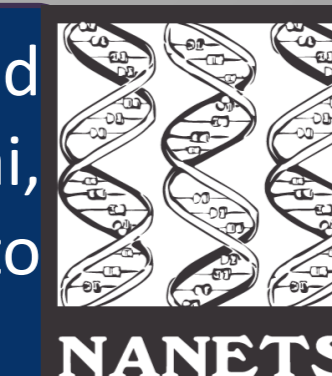
We have previously found that Vandetanib stops tumor growth in an animal model for MTC by an antiangiogenic effect. Interestingly Vandetanib had little effect on cancer cell proliferation *in vivo*. Therefore we hypothesized that combining Vandetanib, or another TKI that has a mode of action similar to that of Vandetanib, with an antiproliferative can stop MTC growth.

Here we investigate Nintedanib and the HDAC inhibitor, Romidepsin, as monotherapies in a preclinical MTC mouse model. We evaluate subsequently Nintedanib/Romidepsin as a combination therapy for MTC. Furthermore we assess a new magnetic resonance imaging (MRI) technique, Amide Proton Transfer (APT), as an imaging biomarker for the prediction of therapeutic response.

Conclusions

- The NSE/p25-GFP mouse model can be used for the preclinical testing of anticancer therapies and in particular to evaluate possible MTC therapies.
- MTC are sensitive to TKI that exhibit antiangiogenic effects.
- Nintedanib slows tumor progression by targeting tumor vasculature. In addition, Nintedanib decreases MTC cell proliferation and inhibits the mTor pathway.
- Romidepsin exhibits strong antiproliferative effects *in vitro* but not *in vivo*. Combining Romidepsin with Nintedanib alone does not improve MTC treatment.
- Nintedanib is a better MTC treatment when used as a monotherapy rather than as a combinatorial therapy with Romidepsin. However resistance develops overtime.
- The APT signal correlated with Ki67 but not CD31 signal.
- Changes in APT signal were detected in combination therapy-treated animals, 1 week earlier as with conventional MRI .

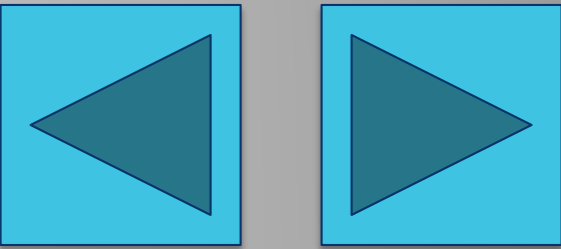
We thank Drs E. Grubbs and J. Fugimoto for staining of a human MTC patient sample, Drs K. Kanganasmi and S. Zhang for MRI scans, R. Telange for technical assistance, J. E. Johnson and J. D. Minna for current mentoring and members of each laboratories for constructive comments. The NSE/tTA and TetOp/p25-GFP mice lines were from Drs E. Nestler and L.H. Tsai, respectively. This research was supported by a NANETS Early Career Development Award to KP, the American Cancer Society MEN-2 Consortium research grants (RSGM-11-190-01 to J.A.B.), UTSW and the NIH. The authors have no conflict of interest to disclose.



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Materials and Methods

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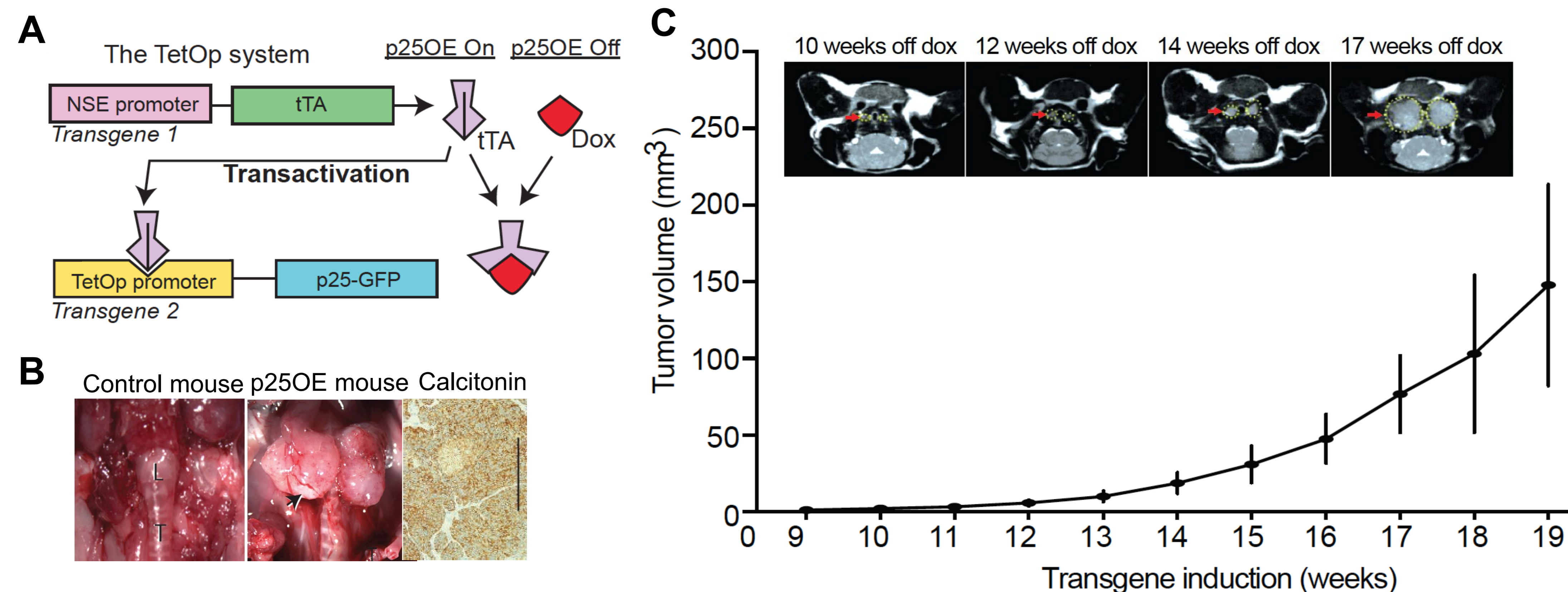


Experimental Design

We used the NSE/p25-gfp bi-transgenic MTC mouse model. Mice were dosed daily intraperitoneally for 3 weeks with either Nintedanib (100 mg/kg/day) or Romidepsin (0.75 mg/kg/day) or [Nintedanib (35 mg/kg/day) + Romidepsin (0.37 mg/kg/day)] or vehicle. Tumor progression was monitored weekly using T2W imaging on a 7T system. APT was performed on a single 1-mm-slice delineating the tumor maximum diameter. Proliferation and microvessel density were determined by immunostaining fixed tumors with Ki67 and CD31 antibodies, respectively. Frozen tumors were analyzed for oncogenic signaling pathways by immunoblotting. Drugs were purchased from LC Laboratories and Tocris R&D and antibodies from Cell Signaling Technology.

Tumor growth in a mouse model for MTC can be monitored in vivo by MRI

(A) Schematic of the bi-transgenic system used to derive the MTC mouse model. p25-GFP overexpression (p25OE) is driven by the Neuron Specific Enolase (NSE) promoter in a Tet-off expression system. (B) p25OE causes the growth of calcitonin-positive thyroid tumors. (C) In vivo monitoring of tumor growth by magnetic resonance imaging (MRI). Mice were imaged every week starting 9 weeks following the induction of p25-GFP expression and thyroid tumor volumes were measured. Red arrow points to the tumor.



Results

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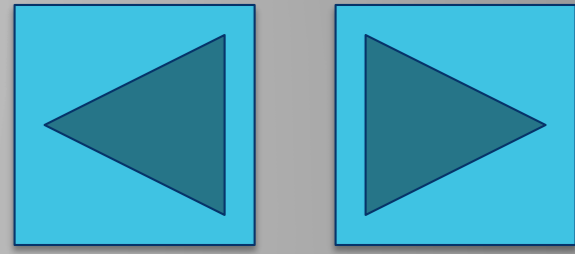
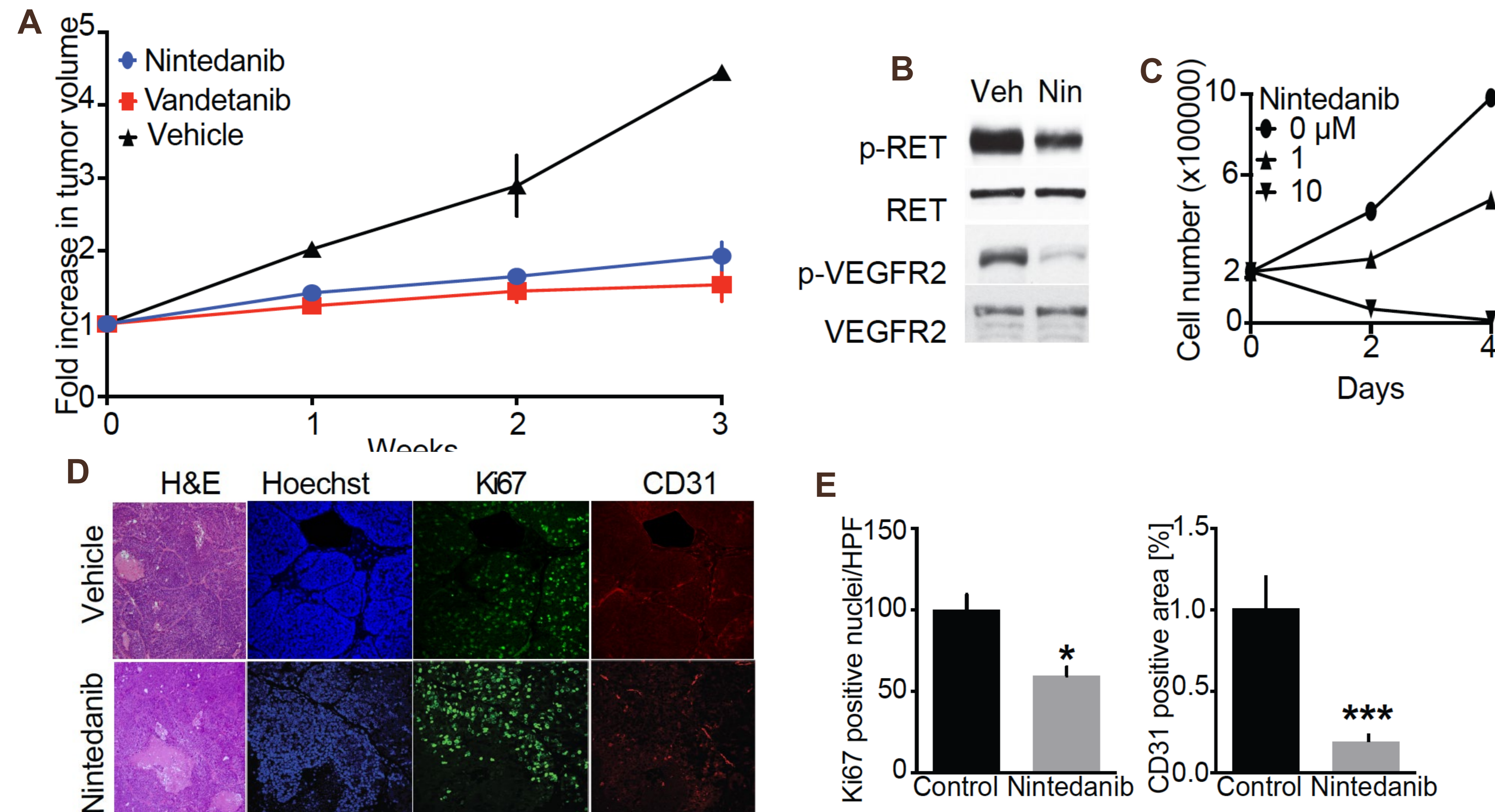


Figure 1. Effect of Nintedanib on tumor growth



(A) Effect of Nintedanib (100 mg/kg/day, oral gavage) vs. Vandetanib (25 mg/kg/day, oral gavage) on mouse MTC growth over a 3-week treatment. (B) Analysis of RET and VEGFR2 activation by immunoblotting. Vehicle (Veh), Nintedanib (Nin). (C) Effect of Nintedanib on cultured mouse MTC cell proliferation. (D) Effect of Nintedanib on proliferation (Ki67 staining) and microvessel .

Results

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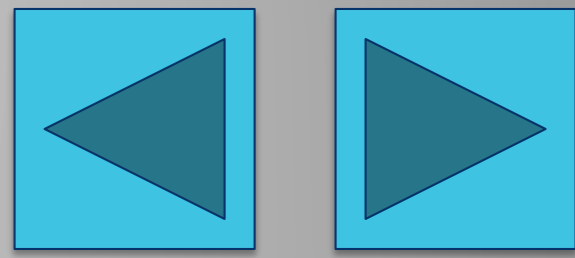
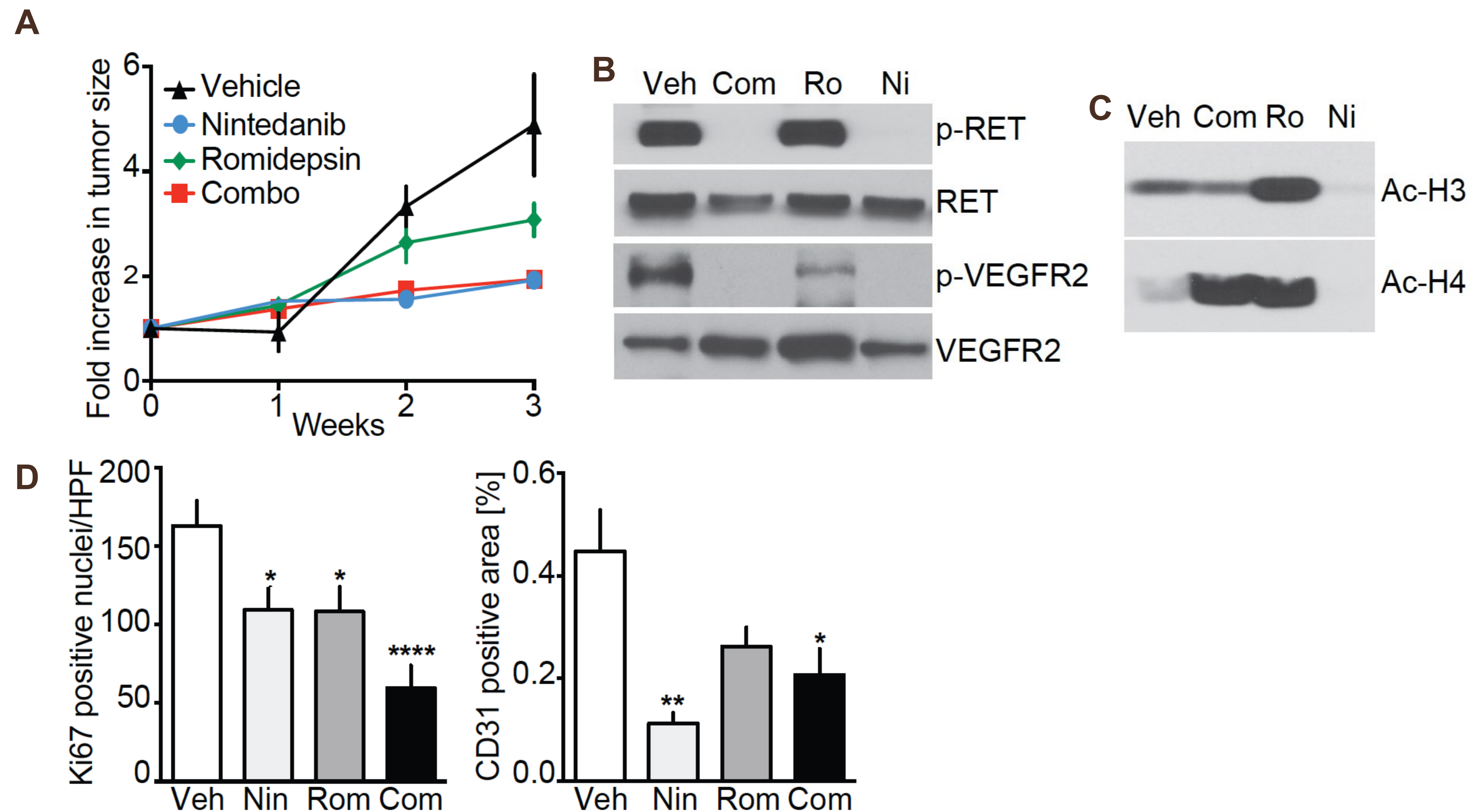
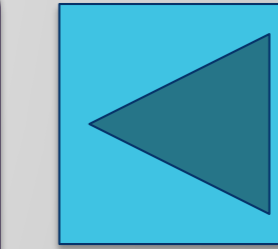


Figure 2. Effect of Romidepsin on tumor growth



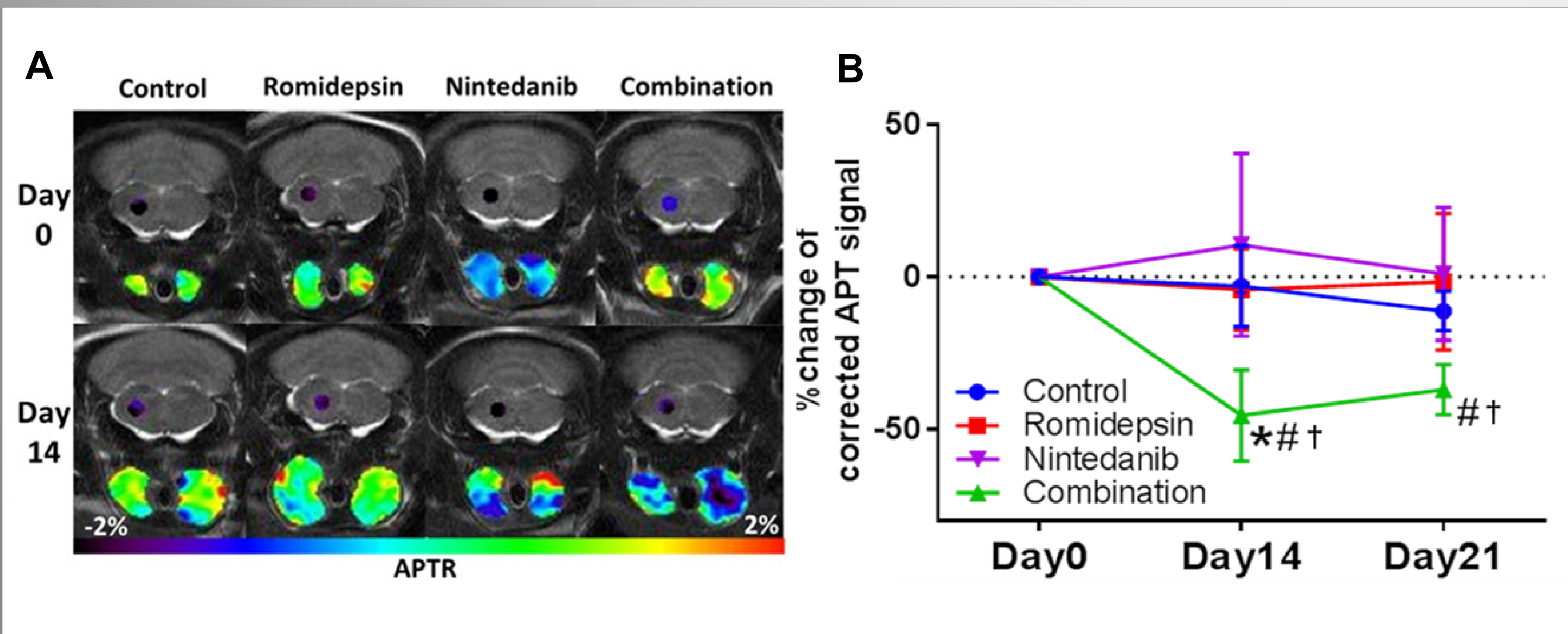
(A) Effect of Romidepsin (0.78 mg/kg/day, i.p.), Nintedanib (35 mg/kg/day, i.p.), Combo (Romidepsin 0.35 mg/kg/day, i.p. + Nintedanib 35 mg/kg/day, i.p.) on mouse MTC growth. (B) Immunoblot analysis of mouse tumors for RET and VEGFR2 activation and (C) for histone acetylation. (D) Analysis of the effect of TKI-based treatments on proliferation (Ki67 staining) and microvessel density (CD31 staining). Analyses in (B-D) were conducted after a 3-week drug treatment. Vehicle (Veh), combinatorial therapy (Com), Romidepsin (Ro), Nintedanib (Nin).

RESULTS



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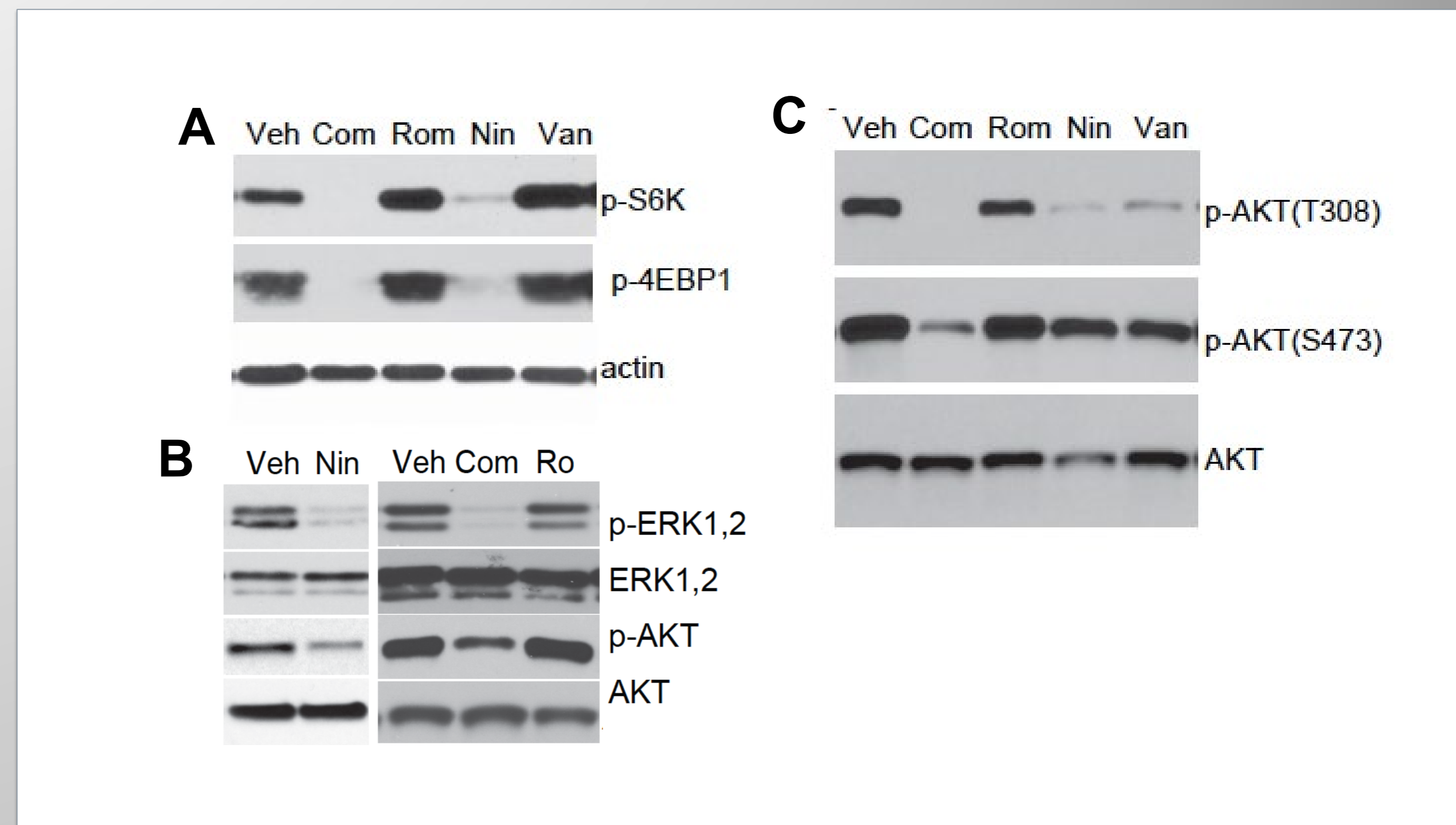
Figure 3. Amide Proton Transfer imaging can detect anticancer effect at least 1 week before MRI



(A) Typical APT-weighted images of the control, the romidepsin, the nintedanib and the combination groups at Day 0 and Day 14. Tumors showed higher APT signal than normal brain stems. The APT signal of tumors obviously decreased in the combination group while it stayed constant in the other three groups

(B) Temporal change (%change) of corrected APT signal (MTR_{asym} at 3.5 ppm) relative to that at Day 0. The APT signal substantially reduced at Day 14 only in the combination group. *: < 0.05 vs. control, #: < vs. romidepsin, †: < 0.05 vs. nintedanib by Tukey's multiple comparison test.

Figure 4. TKI-based treatments affect the PI3K/AKT, mTor and MAPK signaling pathways



Immunoblot analysis of (A) mTor (p-S6K, p-4EBP1), (B) MAPK (p-ERK1,2) and (C) PI3K/AKT (p-AKT) signaling pathways in mouse tumor lysate mouse after a 3-week treatment with a TKI-based therapy. Vandetanib (Van), 25 mg/kg/day, oral gavage (A,C), Nintedanib (Nin), 100 mg/kg/day, oral gavage (A); 35 mg/kg/day i.p (C)), Combo (Com), Romidepsin 0.35 mg/kg/day, i.p. + Nintedanib 35 mg/kg/day, i.p. (B,C), Romidepsin (Ro), 0.35 mg/kg/day, i.p. (B). Veh, Vehicle.