

Systemic delivery and antitumor activity of siRNA complexed with Single Walled Carbon Nanotubes

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Abstract

siRNA is a potential new cancer therapy with the prospect of wide target applicability and high specificity, but currently lacks an adequate delivery mechanism. Ensysce Biosciences has been exploring the use of single-walled carbon nanotubes (SWCNT) as a delivery vehicle for siRNA and previously reported on the safety of SWCNT itself when delivered in a large dose of 100 µg to mice (equivalent to 30 mg for humans) and in vivo efficacy of SWCNT/siRNA complex. Presented here is data demonstrating that SWCNT can function as universal delivery vehicles for siRNA *in vivo*, producing multi-day target knockdown from a single systemic injection, and producing antitumor activity. The data confirm that SWCNT protect siRNA from enzymatic breakdown in serum, they deliver siRNA through cell membranes and that the SWCNT complex can carry multiple siRNAs. A study was conducted to evaluate the systemic delivery of SWCNT/siRNA in tumored animals. The SWCNT complex was prepared using solutions of siRNA and PEG providing solutions containing up to 184 mg/L SWCNT/siRNA carrying siEGFR or siKRAS as single payloads or together as dual payloads. The final total delivery of siRNA ranged from 20 to 40 µg for these proof of principle studies, to examine the ability of SWCNT to deliver the payloads systemically. Female nude mice bearing MiaPaCa-2 tumors were administered 35 µg total dose of SWCNT i.v. by tail vein injection with control groups receiving no treatment, the dual siRNA in vehicle alone, SWCNT/siRNA or SWCNT/dual siRNA. Two mice from each treatment group were sacrificed at 1 week following single injection and the remaining animals (8 per group) received 4 weekly i.v. injections of the SWCNT complexes. Tumor volumes and animal weights were measured twice weekly for 4 weeks. At 24, 48, 72 and 96 hrs after the final treatment, organs and tumors were excised, flash frozen and later evaluated for protein levels by Western Blotting. Animals in the SWCNT treatment groups did not show any weight loss or hematology or blood chemistry changes from normal. MiaPaCa-2 has mutant KRAS. Significantly, the SWCNT complex with siKRAS alone, and with both siKRAS and siEGFR showed a growth delay of 10 days within a 4 week period with once-weekly injections. Tumor EGFR and KRAS proteins levels were measured in tumor at 24, 48 and 72 hr. In conclusion, we have shown that SWCNT can be used to safely deliver siRNA, and siRNA targeted to KRAS, a cancer target for which there is currently no therapy, produces tumor growth inhibition.

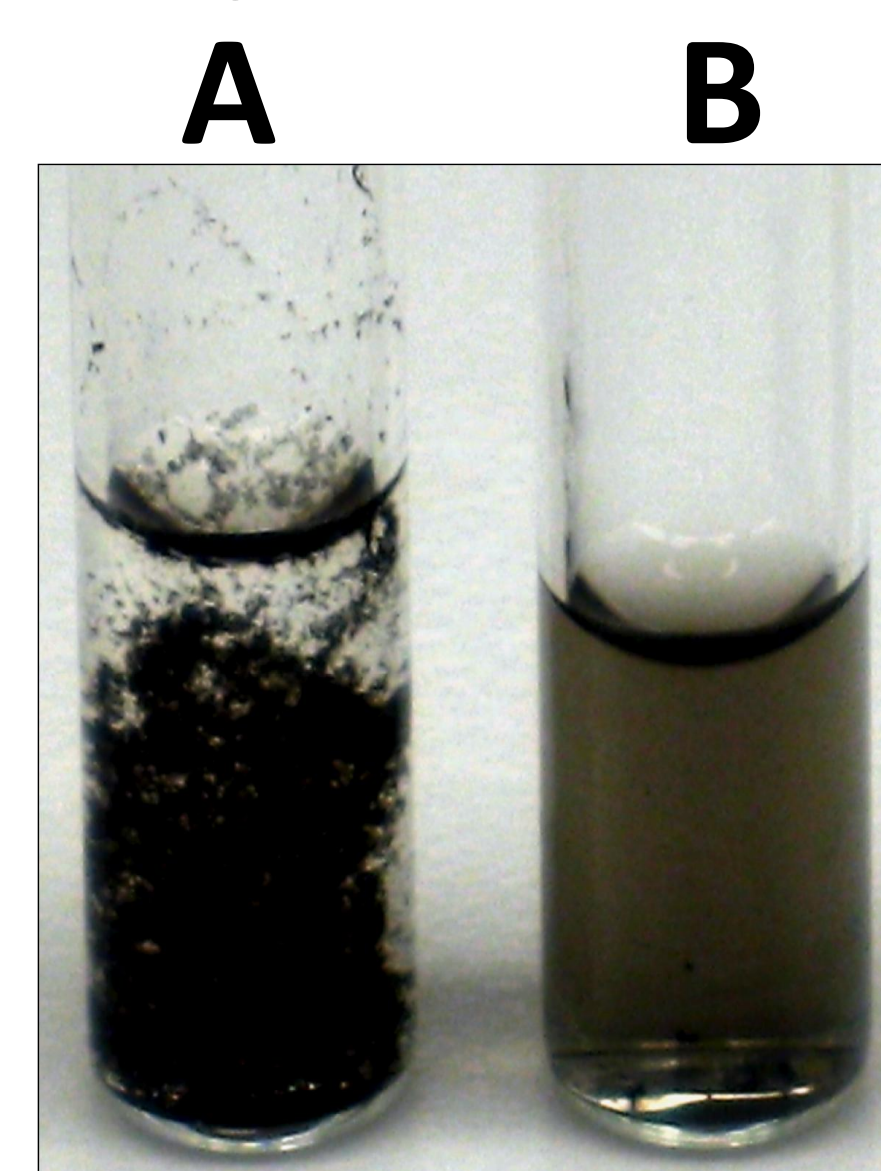


Figure 1: SWCNTs can be solubilized by complexing with siRNA in an aqueous solution. SWCNT in (A) buffer remains insoluble while in siRNA (B) forms a soluble complex.

Methods

Preparation of SWCNT/siRNA complexes: As-synthesized raw HiPco SWCNTs (lot HPR 188.4) was dispersed in solutions of siRNA to provide solutions of up to 184 mg/L SWCNT/siRNA or SWCNT/dual siRNA. Solutions were cleared of residual catalytic metal particles, carbon nanotube bundles and other solid impurities. The resultant solution was collected and the nanotube concentration determined via fluorescent measurement using a NanoSpectralyzer N2. The SWCNT concentration used for the animal study was 35 mg/mL.

Animal studies: Eight to ten-week-old female athymic nude (nu/nu) mice (Harlan) were inoculated s.c. in the right flank with 10^7 MiaPaCa-2 cells per 0.1mL PBS. After tumor initiation and growth, animals were randomized to groups of 10 mice with tumors averaging 100 mm³. SWCNT/siRNA solutions were administered as a single i.v. dose of 35 µg SWCNT in approximately 200 µl either weekly or twice weekly. The 5 groups were: No treatment control (once-weekly injections only), Vehicle control (dual siRNA/PEG solution), SWCNT/siEGFR, SWCNT/siKRas, and SWCNT/dual siRNA. At 24hr (once and twice-weekly injections), 48, 72, 96 hr (once-weekly injections) animals were sacrificed and tumor, organs and blood were collected for routine hematology (CBC and differential) and chemistry including creatinine, BUN (blood urea nitrogen), AST (SGOT), and ALT (SGPT). Macroscopic observations were performed at necropsy to check for presence of SWCNTs in the IP cavity and/or on any organs. Tumor and organs (brain, heart, lung, liver, spleen, and kidneys) were harvested and flash frozen for future analyses.

Animal experiments were approved by the Institutional Animal Care and Use Committee and conducted in accordance with Institutional guidelines.

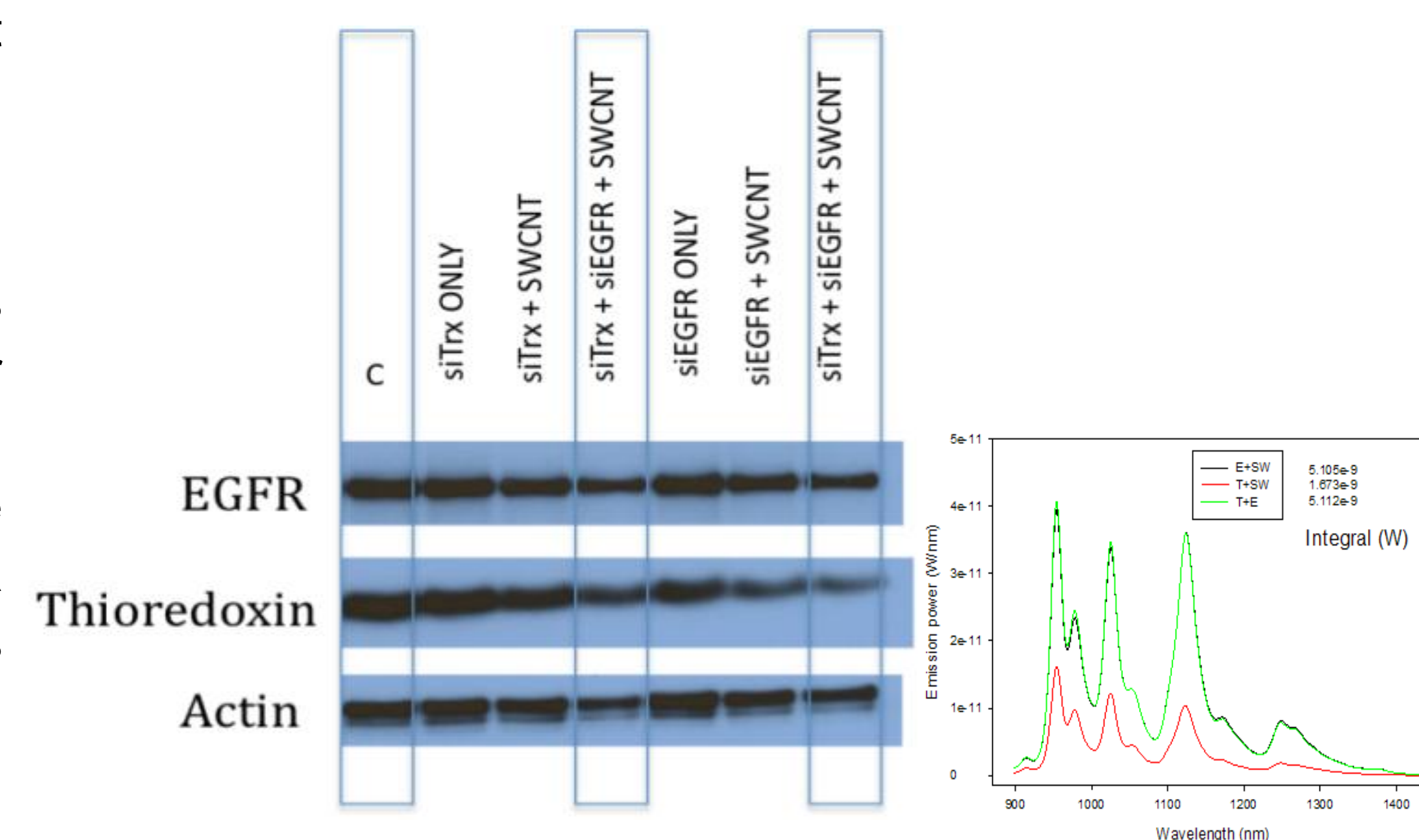


Figure 2: *In vitro* efficacy of dual payload SWCNT/siRNA. MiaPaCa-2 cells in culture were exposed to SWCNT complexed to siTrx and siEGFR for 24 hr, washed and incubated for 72 hr and Western blotted for target proteins. Dual payload reduced Trx protein by 28% and 63%, and EGFR by 16% and 33% in the dual lanes respectively.

Results

Tumor target knockdown at 24, 48 and 72 hour after single injection

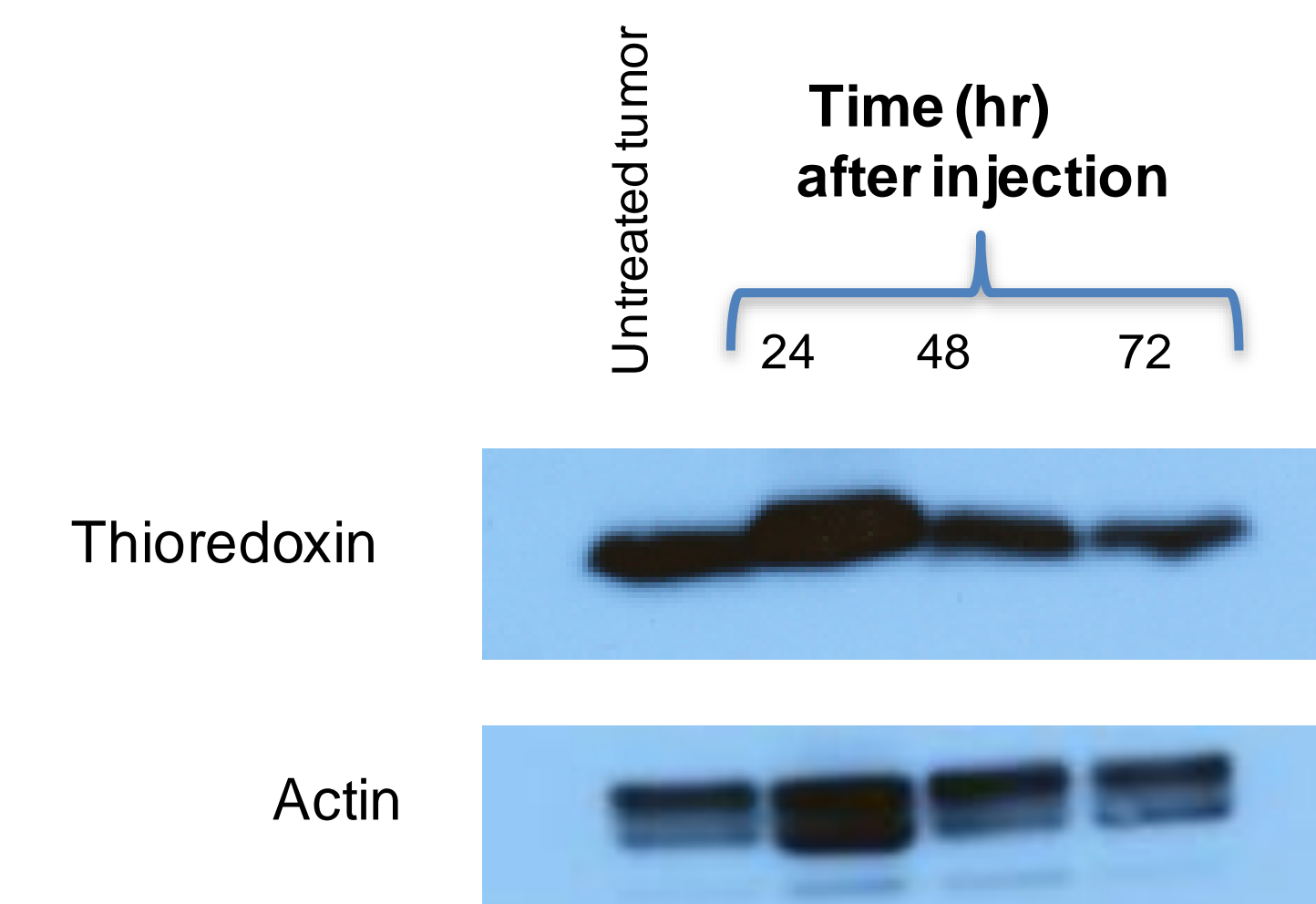


Figure 3: *In vivo* target knockdown in mice receiving a single i.v. bolus of 50 µg SWCNT/siTrx. At 72 hr target protein was reduced by 30 to 40%. Proof of principle study examining SWCNT/siRNA delivery.

Antitumor activity of SWCNT/siRNA dual payload delivered systemically weekly over 4 week period

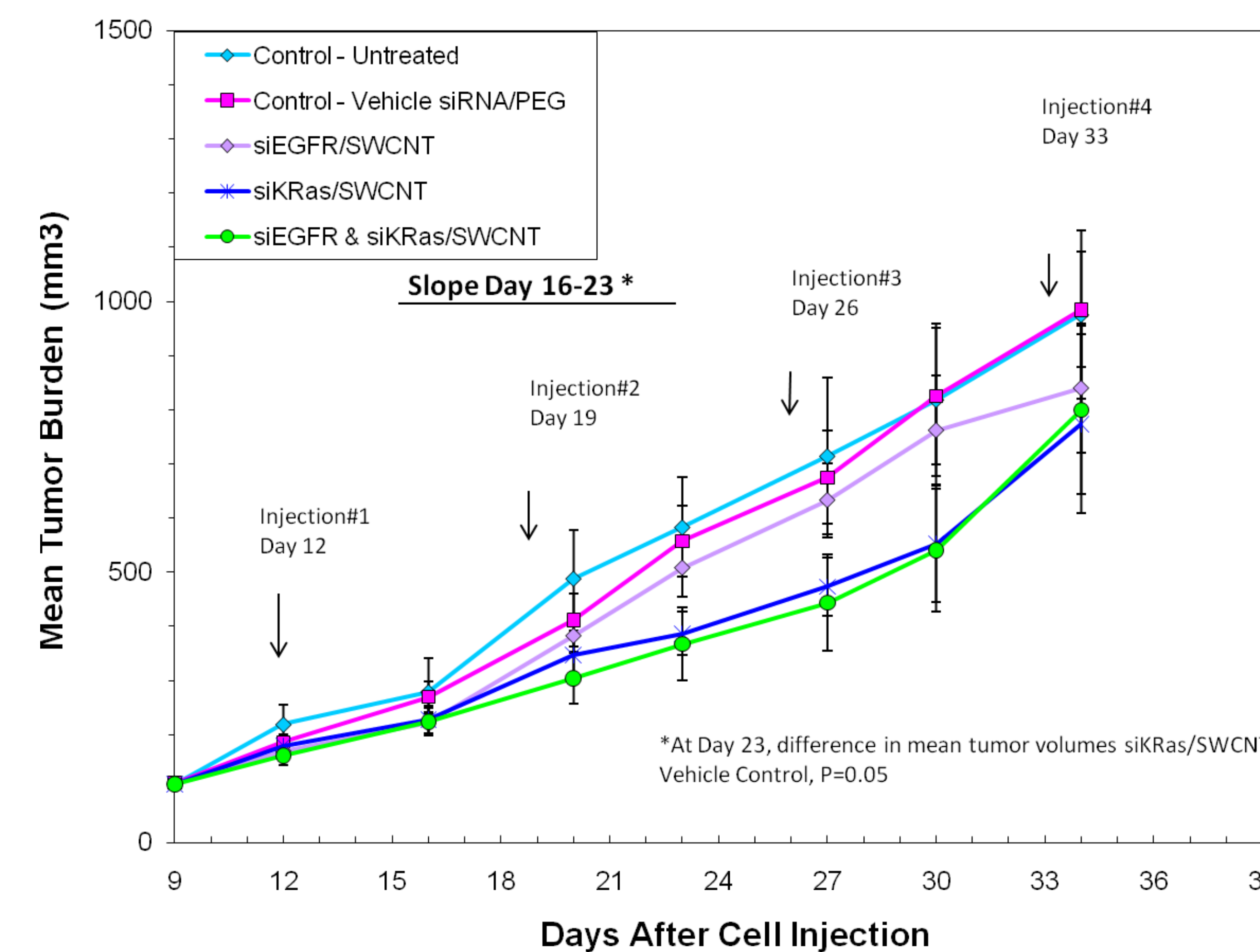
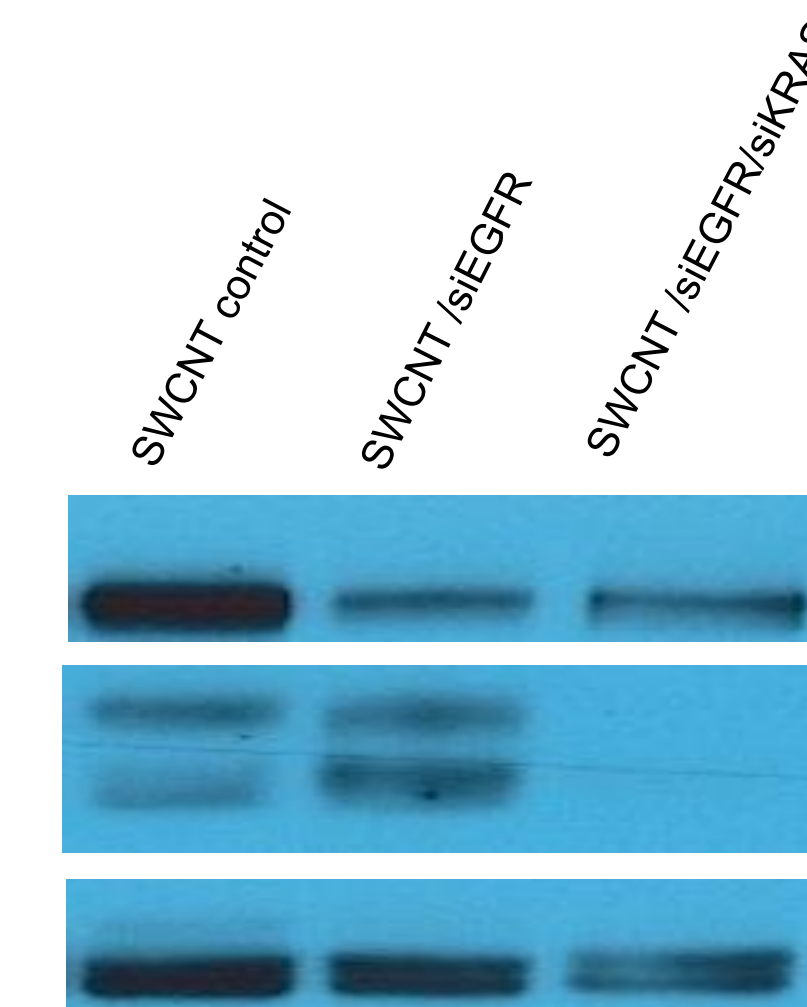


Figure 4: Human pancreatic MiaPaCa xenografts showed growth delay of 10 days with weekly treatment. When tumors reached 100 mm³ mice received a weekly injection of 35 µg SWCNT solubilized with siRNA, or vehicle with siRNA alone. Tumors were measured twice weekly. N=8



Tumor protein target knockdown at 96 hr

Figure 4a: MiaPaCa-2 tumors from weekly study, Western blotted for EGFR and KRAS 96 hr following 4th treatment.

Twice weekly systemic delivery of SWCNT/siRNA dual payload

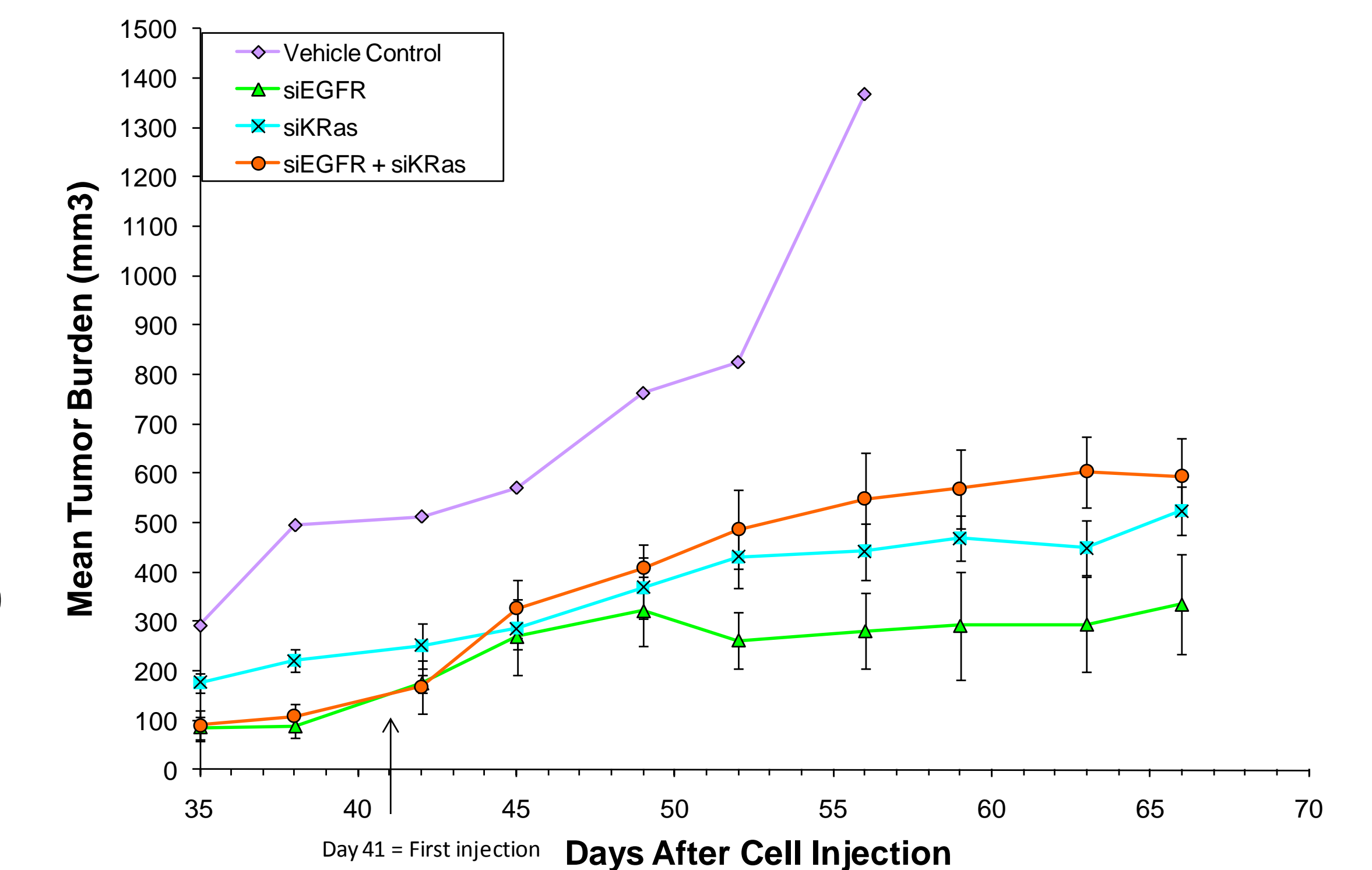


Figure 5: Solutions of siRNA targeting EGFR, KRAS or both complexed to SWCNT produced significant growth delay when delivered twice weekly. SWCNT complexes were injected via tail vein of mice with MiaPaCa-2 human pancreatic tumors twice weekly for 4 weeks. Tumor volumes were measured twice weekly. N=5-6 for treatment groups.

Conclusions

- No signs of toxicity including hematology, blood chemistry, or macroscopic observations were observed in mice following a large i.v. dose of optimized preparation of SWCNT solubilized with siRNA delivered weekly or twice weekly for 4 weeks.
- Dual payload siRNA complexes provide knockdown of multiple target proteins.
- Significant antitumor activity was provided by both weekly and twice weekly delivery of SWCNT/siRNA complexes against MiaPaCa-2 human pancreatic tumor xenografts.
- Twice weekly administration of nanotube delivered siRNA appears to provide excellent tumor control.