OMTO, Volume *22*

Supplemental information

A novel protein-drug conjugate, SSH20, demonstrates significant efficacy in caveolin-1-expressing tumors

Ryan Robb, Jimmy Chun-Tien Kuo, Yang Liu, Sergio Corrales-Guerrero, Tiantian Cui, Ahmad Hegazi, Gregory Nagy, Robert J. Lee, and Terence M. Williams

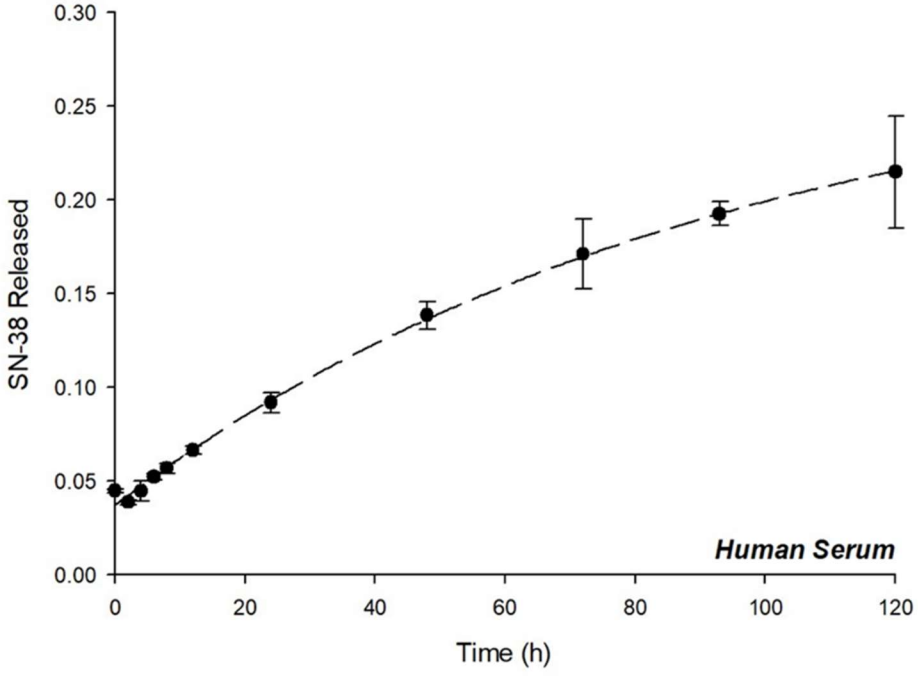
Supplementary material

Methods

***In vitro* serum stability.** Briefly, 180μL of SSH20 was added to 1620μL of pooled human serum off the clot (Innovative Research, Novi, MI) to form a 10% SSH20 solution, which was incubated at 37°C. Aliquots were collected at preset time points, a fixed amount of internal standard (10-hydroxycamptothecin, 10-HCPT) was added to each sample aliquot and the solutions were mixed thoroughly on vortex prior to protein precipitation by acetonitrile. Protein precipitates were pulled down by 17,000 x g centrifugation for 5 minutes. The dissociated SN-38 in the supernatant was analyzed by reverse-phase HPLC, using parameters described above, detected by PDA at 368nm. SN-38/internal standard peak ratios were correlated with an SN-38 standard curve (using the same preparation method) to obtain the concentrations of released SN-38. The plot of the kinetics of SN-38 release was generated by Prism, and the half-life was calculated by one-phase exponential association.

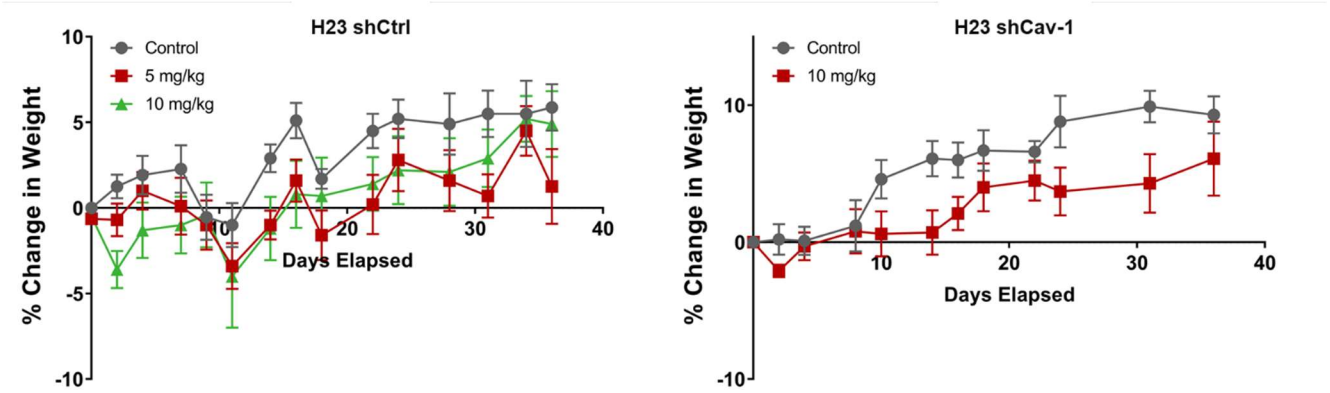
***In vivo* studies.** Animal studies were conducted in accordance with an approved protocol adhering to the Institutional Animal Care and Use Committee policies and procedures at The Ohio State University (Columbus, OH). Eight- to ten-week-old male athymic nude mice (Taconic Farms Inc.) were caged in groups of five or less and fed a diet of animal chow and water ad libitum. H23 cells (2×10^6) with stable control shRNA (shCtrl) or shCav-1 were injected subcutaneously into the flanks of athymic nude mice. Treatment regimens were started once tumors reached approximately 100-200 mm^3 in size (typically 1-3 weeks post-injection). SSH20 (10 mg/kg) was administered intravenously via retro-orbital injection accordingly. Weight and clinical signs of toxicity were monitored several times per week.

Supplementary Figure S1



SFigure S1. Kinetics of SN-38 release from SSH20 incubated in human serum. The half-life was calculated by one-phase exponential association. The release of SN-38 from SSH20 followed a time-dependent manner with a half-life of ~ 64.2 hours.

Supplementary Figure S2



SFigure S2. SSH20 treatment results in no detectable changes in weight compared to vehicle-treated mice. H23-shCtrl/shCav-1 cells were injected into the flanks of mice. Once tumors reached approximately 100-200 mm3 in size, 0.9% saline (vehicle), or SSH20 (5 or 10 mg/kg) was administered intravenously via retro-orbital injection. No significant differences in weight were detected between vehicle or SSH20 treated groups.