Supplementary Data for

Poloxamer-linked prodrug of a topoisomerase I inhibitor SN22 shows efficacy in models of high-risk neuroblastoma with primary and acquired chemoresistance

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This file includes Supplementary Figures S1 to S5.
Fig. S1. The synthetic scheme and $^1$H NMR spectra (400 MHz, CDCl$_3$) of Poloxamer-linked prodrugs of SN22 and SN38. The Poloxamer 338 (Pluronic F-108) scaffold has $m$$\sim$50 and $n$$\sim$135.
Fig. S2. Growth inhibition of MYCN-amplified NB cells exhibiting an acquired loss of p53 tumor suppressor function [BE(2)C] by PF108-[SN22]₂ prodrug vs. SN22 with or without Pluronic F-108 (doses equivalent to 100 nM of SN22). Cells untreated or treated with blank Pluronic F-108 were included as controls. Tested exposure durations included 24 hr and 30 min (A and B, respectively). Cell growth was monitored over time by bioluminescence. Results are shown as mean ± SD.
Fig. S3. ABCG2 transporter expression by NB cells representing different forms of MYCN-driven high-risk disease: MYCN-amplified IMR-32 and BE(2)C cells derived at diagnosis and at relapse after intensive chemoradiotherapy, respectively, and MYCN-overexpressing TH-MYCN NB cells derived from chemo-naïve primary tumors. ABCG2 expression was determined by Western blot densitometry using whole cell extract (50 µg) and a rat monoclonal antibody to ABCG2 (BXP-53, Santa Cruz Biotechnology) diluted 1:500 in 5% BSA/PBS-Tween 20 as a blocking agent. Densitometric analysis was performed with ImageJ software. The results normalized to β-actin are presented as mean ± SD.
Fig. S4. Therapeutic efficacy of PF108-[SN22]₂ in an orthotopic model of chemo-naïve (newly diagnosed) MYCN-amplified NB. Mice were inoculated with $10^6$ IMR-32 cells stably expressing luciferase. Treatment with PF108-[SN22]₂ was administered intravenously at a dose equivalent to 10 mg/kg of SN22 once a week for 4 weeks. Irinotecan administered twice a week at 15 mg/kg over 4 weeks was included as a control. Tumor-associated signal was monitored by quantitative bioluminescence (representative images taken at 0-9 weeks are shown in Fig. 3A of the main text). Quantitative data presented graphically in A are expressed as mean ± SD. The survival curves for respective animal groups are shown in B.
**Fig. S5.** The comparative effect of the prodrug treatment on tumor tissue histology and cell proliferation in a genetically engineered TH-MYCN mouse model of de novo resistant, MYCN-driven NB. PF108-[SN22]₂ (A), PF108-[SN38]₂ (B), or irinotecan (C) were administered intravenously to homozygous TH-MYCN mice with verified large NB tumors at doses corresponding to 10 mg/kg of SN22 or SN38. Tumors were harvested 24 hr post-treatment, formalin-fixed, embedded in paraffin and stained with an antibody to Ki67 (SP6, Abcam) using a Bond Max automated staining system (Leica Microsystems). Slides rinsed and dehydrated with ethanol and xylene were coverslipped and photographed at an original magnification ×200. The numbers of Ki67-positive cells in respective tumor samples (four fields per group) are expressed as mean ± SD (D).