Calaspargase pegol and pegaspargase cause similar hepatosteatosis in mice

Asparaginase has been a backbone of pediatric acute lymphoblastic leukemia (ALL) therapy for decades, and has recently become routinely used in pediatric-inspired regimens to treat older adolescent and young adult (AYA) patients.[1] Unfortunately, asparaginase causes multiple severe toxicities, including hepatotoxicity. Asparaginase-associated hepatotoxicity is common and dose-limiting for concurrent hepatically-metabolized chemotherapy and can result in chemotherapy dose reductions and/or omissions. Severe hepatotoxicity during early chemotherapy is associated with 10-30% reductions in event-free survival.[2,3] Obesity, which is present in 20-33% of newly diagnosed pediatric and adult ALL patients,[4,5] is associated with a higher risk of hepatotoxicity,[5,6] further complicating incorporation of asparaginase into frontline therapy.

Until recently, pegaspargase (Oncaspar; SS-PEG) constituted the frontline pegylated L-asparaginase product for pediatric and AYA ALL. However, in December 2022, SS-PEG was removed from the US market for patients <22 years old in favor of calaspargase-pegol-mknl (Asparlas; SC-PEG). SC-PEG received regulatory approval in 2018 to replace SS-PEG based on two trials, DFCI 11-001 and COG AALL07P4.[7,8] With a different linker molecule, SC-PEG has a longer half-life and an approximately 50% higher overall exposure (by area-under-the-curve).[7] Though not completely elucidated, asparaginase-induced hepatotoxicity is associated with hepatosteatosis.[9] Increased asparaginase exposure has therefore created concerns for correspondingly greater hepatotoxicity, particularly in those with obesity and in AYA. However, the two head-to-head trials of SC-PEG *versus* SS-PEG were neither designed nor able to conclusively address this question of increased hepatotoxicity. Both trials included a majority of patients less than 10 years old, who are at baseline lower risk of hepatotoxicity. Also, they neither assessed obesity, nor reported clinically-relevant thresholds, such as hyperbilirubinemia requiring dose-modification.[7,8] In the COG AALL07P4 trial, a significantly increased risk for all-grade hepatotoxicity from SC-PEG was indeed found in a late treatment phase. This difference was not present when limited *post hoc* to grade ≥3, possibly due to the population and/or small numbers in the trial.[7] Despite SC-PEG approval in 2018, frontline use was limited until the recent removal of SS-PEG from the market for patients <22 years of age. In the absence of rigorous data for comparable safety, we therefore sought to use *in vivo* models to investigate this concern for hepatosteatosis. We hypothesized that SC-PEG would exhibit greater hepatosteatosis in a mouse model with and without obesity. To test this, we performed a side-by-side comparison of SC-PEG *versus* SS-PEG in control and obese mice to

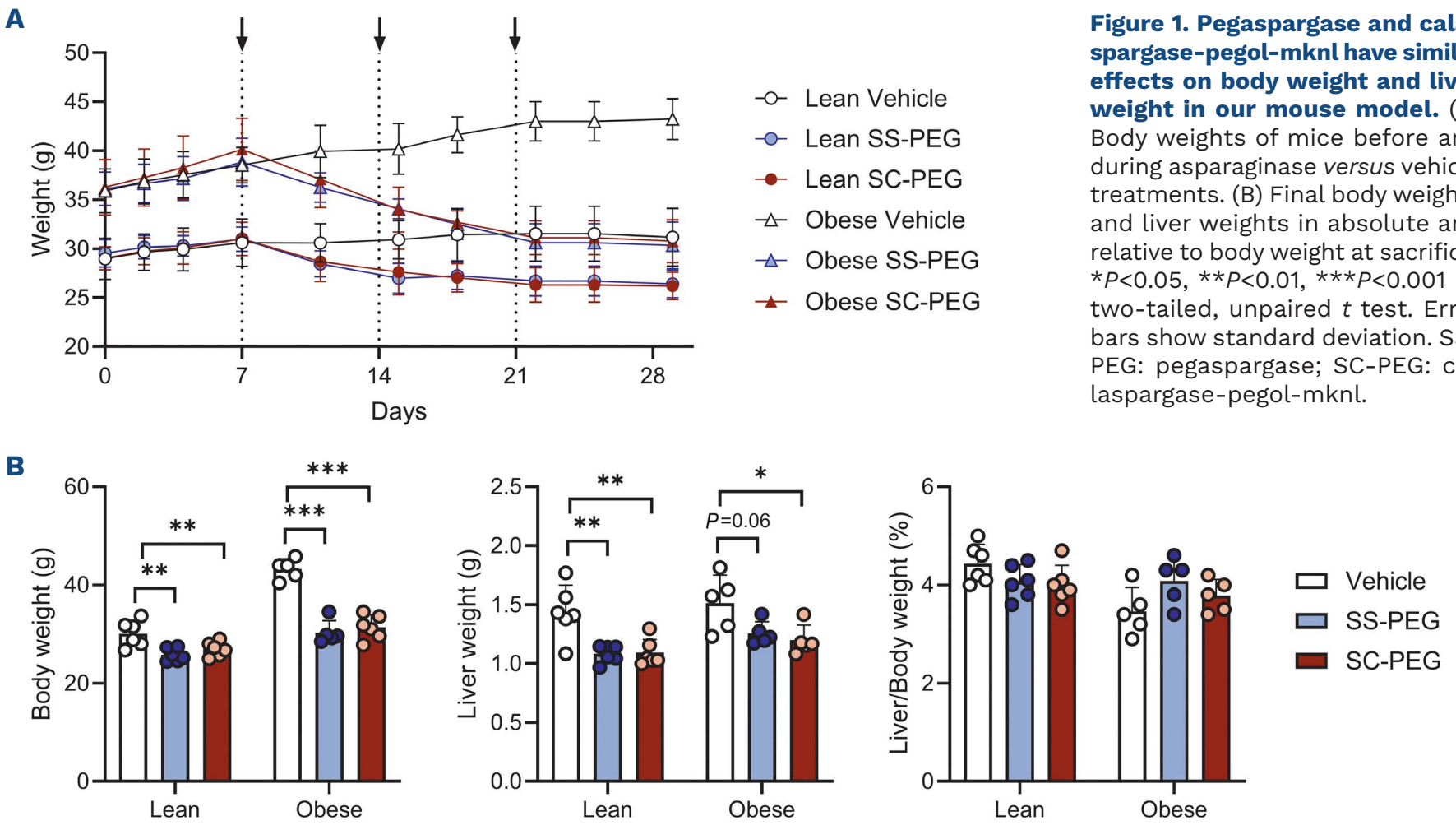
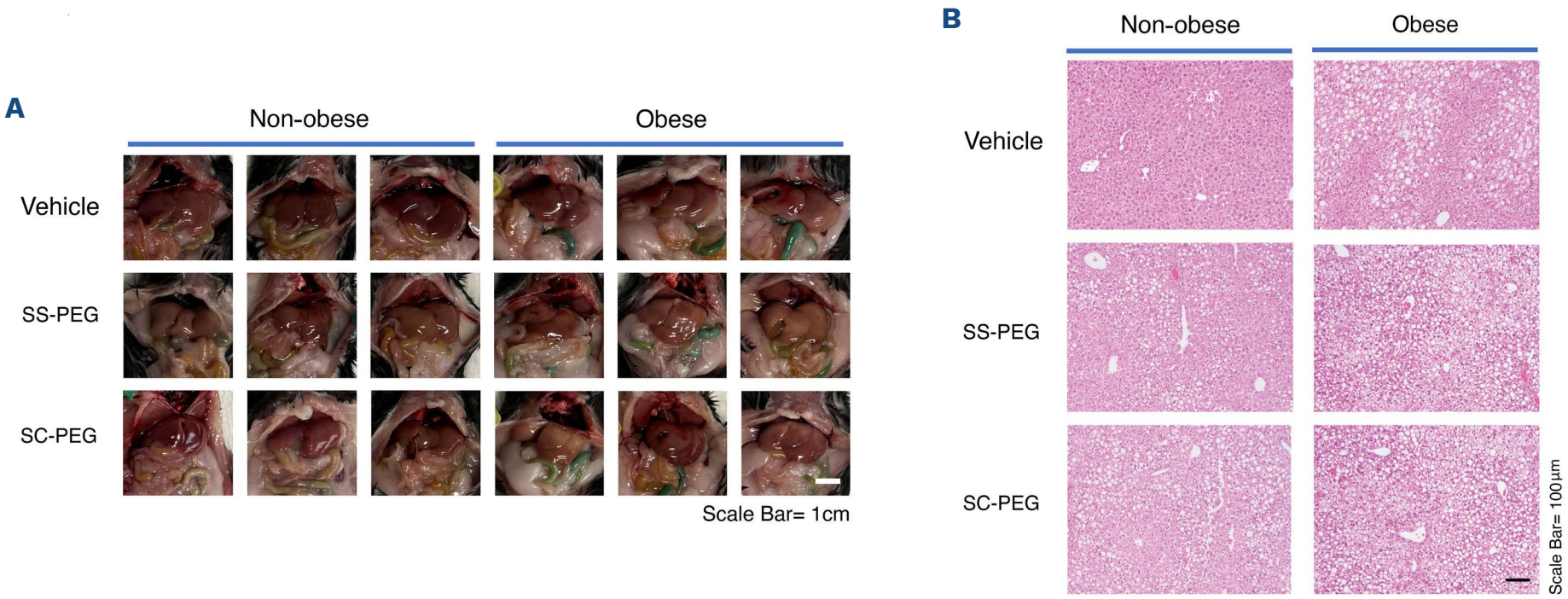


Figure 1. Pegaspargase and calaspargase-pegol-mknl have similar effects on body weight and liver weight in our mouse model. (A) Body weights of mice before and during asparaginase *versus* vehicle treatments. (B) Final body weights and liver weights in absolute and relative to body weight at sacrifice. **P*<0.05, ***P*<0.01, ****P*<0.001 by two-tailed, unpaired *t* test. Error bars show standard deviation. SS-PEG: pegaspargase; SC-PEG: calaspargase-pegol-mknl.

evaluate differences in hepatosteatosis and hepatotoxicity. Eighteen 15-week-old male diet-induced obese (DIO) C57BL/6NTac mice (DIO-B6-M, Taconic, La Jolla, CA) and 18 control mice (DIO-B6-Control) received weekly intraperitoneal injections of 1,000 IU/kg SC-PEG, 1,000 IU/kg SS-PEG, or vehicle (N=5-6 per group). All mouse work was approved by the UCLA Institutional Animal Care and Use Committee (IACUC approval ARC-2017-053) and performed in accordance with the USPHS Policy on Humane Care and Use of Laboratory Animals. Asparaginase-treated mice exhibited significant weight loss, with obese mice losing ~8 grams and control mice losing ~4 grams of body weight during the 3-week treatment period (Figure 1A). There were no differences in the weight curves between the two asparaginase formulations. Mice were humanely euthanized with CO_2 inhalation 7 days after the third injection. Liver weight decreased with asparaginase treatment, though when normalized to body weight there were no significant differences (Figure 1B). Grossly, livers from the DIO mice were more tan in appearance compared to control mice (Figure 2A). Both asparaginase formulations caused livers from control mice to take on a more tan appearance, and livers from DIO mice to become more pale yellow, with no visible difference between SS-PEG and SC-PEG. Sections



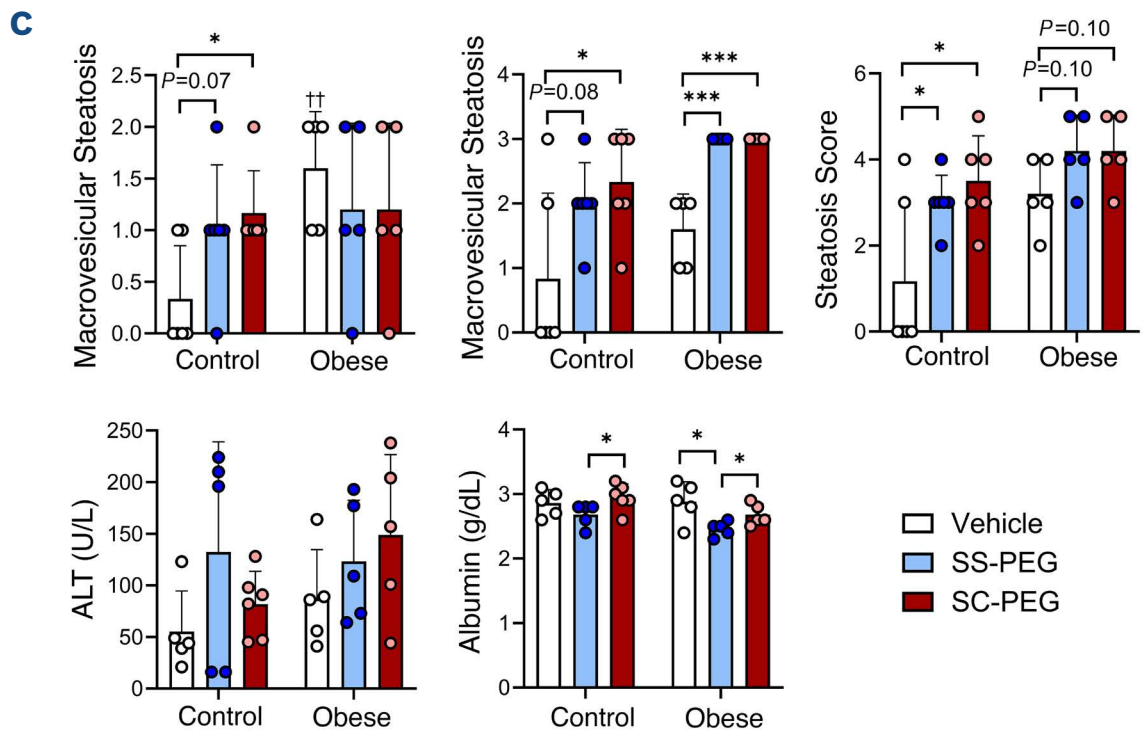


Figure 2. Pegaspargase and calaspargase-pegol-mknl lead to similar hepatosteatosis in our mouse model. (A) Photos of livers *in situ* after asparaginase *versus* vehicle treatment. (B) Representative images of hematoxylin and eosin-stained liver sections, taken on an Olympus BX43 microscope at 20x magnification with a 0.4 numerical aperture at 22°C. Image was acquired using Olympus DP27 Acquisition software Olympus cellSens Standard 3.2 (Build 23706), with no further image processing. (C) Steatosis scores and serum measures from mice 3 days after asparaginase or vehicle injections. *P<0.05, ***P<0.001 as indicated. ††P<0.01 *versus* control vehicle condition. All comparisons by two-tailed, unpaired *t* test. Error bars show standard deviation. SS-PEG: pegaspargase; SC-PEG: calaspargase-pegol-mknl; ALT: alanine aminotransferase.

of the right and left medial lobes were fixed and stained with hematoxylin and eosin (H&E) for evaluation of steatosis (Figure 2B). DIO mice showed baseline steatosis, which was increased by both asparaginase formulations. Images were examined by a board-certified pathologist (JJC) blinded to the treatment conditions and scored for steatosis and inflammation per an established murine scoring system.[10] Vehicle-treated DIO mice showed higher macrovascular steatosis than control vehicle mice (Figure 2C). Asparaginase increased macrovascular steatosis in control mice, and microvascular steatosis in both diet groups, with no difference between the two asparaginase formulations. Steatosis scores increased in the control mice and showed a tendency to increase in DIO mice. Serum alanine transaminase (ALT) was not significantly increased by either asparaginase treatment. Serum albumin levels declined with treatment, more so with SC-PEG than SS-PEG (P<0.001 in controls, P<0.05 in obese, two-sided t test).

In the present study, we demonstrate that both SS-PEG and SC-PEG cause hepatosteatosis in our mouse model, but there were no clear differences between these two formulations at equivalent dosing. SS-PEG caused a further decrease in plasma albumin than SC-PEG, thought the net difference was modest. Neither formulation resulted in transaminitis in the present study. Though elevated liver enzymes and bilirubin have been reported after a dose of 1,500 IU/kg of SS-PEG,[11] our findings are consistent with the majority of studies that reported no transaminitis in mice treated with native asparaginase[12-14] or even after four doses of up to 5,400 IU/kg SS-PEG.[15] While this preclinical *in vivo* assessment of relative hepatotoxicity is reassuring for ongoing use of SC-PEG, the use of an adult DIO mouse model cannot definitively predict effects in children and AYA patients. Future real-world clinical studies are needed to assess whether differences in hepatotoxicity arise due to the longer half-life of SC-PEG in patients. Current active trials investigating asparaginase hepatotoxicity (*clinicaltrials gov. Identifier: NCT05602194*), and the ongoing SC-PEG trial in AYA (*clinicaltrials gov. Identifier: NCT04817761*), stratify by obesity and/or focus on dose-limiting hepatotoxicity, and will therefore be critical to further evaluate potential differences in clinically relevant hepatotoxicity between these two asparaginase formulations. Understanding potential differences in asparaginase metabolism both with and without obesity has important clinical implications for determining optimal dosing in children and AYA patients.

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Contributions

VR-T, JJC, MC, and J.Tan performed research. TR and J.Tinajero contributed reagents. VR-T, EO, and SDM designed research. EO and SDM analyzed data and wrote the paper.

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Data-sharing statement

The dataset generated during the current study is available in the Dryad data repository: https://doi.org/10.5061/dryad.2bvq83c0m.

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