Characterization of Rice-Derived Proinsulin-Transferrin Fusion Protein and Its Oral Bioavailability as a Basal Insulin Analog
Y-S. Chen\textsuperscript{1}, J. Shao\textsuperscript{1}, J. Zaro\textsuperscript{1}, D. Zhang\textsuperscript{2}, W-C. Shen\textsuperscript{1}
\textsuperscript{1}University of Southern California, \textsuperscript{2}Ventria Bioscience

Purpose
Our previous studies using HEK293 cell-produced proinsulin-transferrin fusion protein (HEK-ProINS-Tf) have demonstrated that this recombinant protein is a liver-targeted insulin analog and can be used to selectively inhibit hepatic glucose production in type 1 diabetic (T1D) mice. Recently, we have successfully applied a transgenic rice (Oryza sativa L.) expression system to produce the fusion protein, i.e., Rice-ProINS-Tf, and have demonstrated that Rice-ProINS-Tf exhibited a similar characteristics and bioactivity as HEK-ProINS-Tf. The purpose of the current study was to further confirm Rice-ProINS-Tf as an insulin analog for the control of the basal blood glucose (BG) in a T1D mouse model. In addition, the oral bioavailability of Rice-ProINS-Tf in mice was also investigated.

Methods
In vivo BG lowering effect of Rice-ProINS-Tf was performed in streptozotocin (STZ)-induced T1D male C57BL/6J mice (Jackson Lab, ME). For the study of the long-term effect on BG, STZ-induced diabetic mice were subcutaneously injected with Rice-ProINS-Tf or Insulin Glargine with an identical dose of 135 nmol/kg. Mice were kept under 8 h fasting/feeding cycle for 48 h after the injection without further treatment. For oral delivery study, mice were orally administered with Rice-ProINS-Tf or insulin (both at 800 nmol/kg) using a gavage needle. The mice were kept under fasting condition for 12 h post-treatment and BG concentration at different timepoints was determined via tail vain using ONE-TOUCH blood glucose meter (Lifescan, CA).

Results
For the subcutaneous injection study, Insulin Glargine group showed a quick onset of hypoglycemia, and the BG levels were declined from 400 to 80 mg/dL at the first hour. The low BG level sustained for 10 h post-injection, and then bounced back to the same level as in the control group after 12 h. On the other hand, Rice-ProINS-Tf group exhibited a slightly slow onset, but the BG level during the fasted cycle was maintained at ~ 100 mg/dL until 48 h post-injection. For oral delivery study, the orally administered Rice-ProINS-Tf (800 nmol/kg) exhibited a decrease of BG, starting from 4 h post treatment until the end of the experiment at 12 h time point. The BG level of the orally-treated mice was maintained at ~100 mg/dL from 6 to 12 h, which was similar to that of mice with subcutaneous injection of Rice-ProINS-Tf at 22.5 nmol/kg. Therefore, the bioactivity of orally administered Rice-ProINS-Tf was estimated approximately 3% of that of subcutaneously injected Rice-ProINS-Tf.

Conclusion
Rice-ProINS-Tf is a potential candidate as an insulin analog for basal insulin replacement in T1D treatment due to its long-lasting and liver-preferential activity. In addition, Rice-ProINS-Tf is also a potential candidate for the development of an orally-bioactive insulin analog to control the basal BG in diabetic patients.