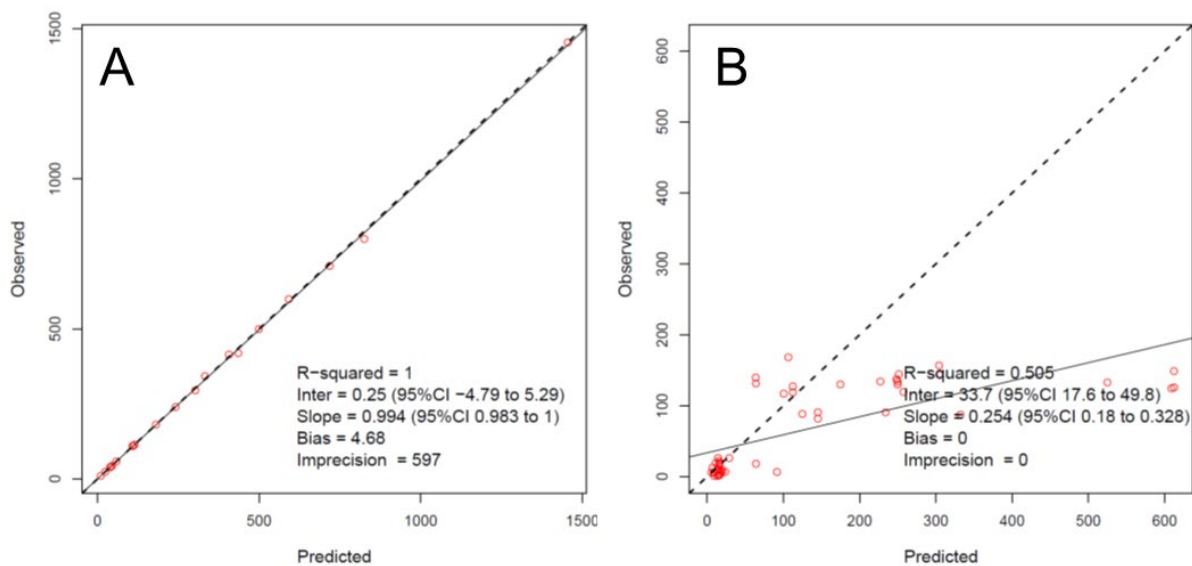


#### sFig 4: Evaluation of long-term storage of serum specimens (NAC assay)

Due to laboratory closures and logistical delays from the COVID-19 pandemic, samples collected to measure NAC concentrations from patients in the expansion cohort were stored for extended periods of time. Samples were analyzed using the identical assay kit in the same laboratory as the previous groups. Observed concentrations for peak NAC in this group were 0.74 mM (range 0.11 – 1.03), markedly lower than NAC concentrations observed at the same dose in DL3. To test the hypothesis of storage degradation, we constructed a population pharmacokinetic model of NAC using Pmetrics, a nonparametric modeling package for R (version 1.52, Laboratory of Applied Pharmacokinetics and Bioinformatics, University of Southern California, Los Angeles, California), employing the algebraic model solver and the nonparametric adaptive grid algorithm. (1) The model included NAC dose, infusion duration, preceding cisplatin dose, age, and body-surface area. In Panel A, the model used data derived from dose-escalation cohort and demonstrates consistent observed vs predicted concentrations. In Panel B, the same model was applied to the dose expansion cohort with long-term sample storage prior to analysis (due to COVID-19 restrictions at the time), which demonstrates observed concentrations consistently lower than predicted, highly likely due to degradation of the samples in storage. This data are shown here for transparency and completeness.



1. Neely MN, van Guilder MG, Yamada WM, Schumitzky A, Jelliffe RW. Accurate detection of outliers and subpopulations with Pmetrics, a nonparametric and parametric pharmacometric modeling and simulation package for R. *Ther Drug Monit* 2012;**34**(4):467-76 doi 10.1097/FTD.0b013e31825c4ba6.