Development of an Immunoassay with Gyrolab Workstation for the Quantification of sAXL in Mouse and Human Plasma
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Purpose
AXL is a member of cell-surface transmembrane receptor tyrosine kinases and is widely investigated as biomarker for certain cancer types. The current method was developed for the purpose of exploring 1) the changes in the levels of soluble AXL (sAXL) in mouse tumorgraft plasma samples after receiving increased amounts of a Teva experimental drug and 2) the basal level of sAXL in plasma from healthy volunteers and cancer patients.

Methods
Recombinant sAXL (a.a. 1–449) was spiked in protein-based buffer to prepare standard and QC samples. Human (NHP), mouse (NMP) and mouse tumorgraft (TMP) plasma samples were used to establish the performance of the method. sAXL present in standards, QCs, mouse and human plasma is captured by biotinylated mouse monoclonal anti-human sAXL antibody onto a Gyrolab Bioaffy CD. The captured sAXL was detected by Alexa Fluor 647-labeled Rabbit polyclonal anti-human AXL antibody. The fluorophore labeled reagent detected by the laser beam is proportional to the amount of captured sAXL. A standard curve is generated using a 5PL fit model. The sAXL plasma concentrations are interpolated from the standard curve, adjusted by dilution factor.

Results
During method development, the minimum required dilution was established at 1:10. NMP samples did not show detectable level of sAXL. TMP collected prior to treatment and NHP samples had variable levels of sAXL from 0 to 5,300 pg/mL. The accuracy and precision of the assay was established using sAXL spiked in protein-based buffer. Nine non-zero standards (15.2 to 100,000 pg/mL) and five levels of QCs spanning from LLOQ (46 pg/mL) to ULOQ (33,333 pg/mL) were tested. Overall inter-assay accuracy and precision varied between 104-116% and 3-13%, respectively. For selectivity 10 individual NMP and 10 NHP samples were spiked at low (100 pg/mL) and HQC (25,000 pg/mL) sAXL levels. Eighty eight % and 100% of the spiked samples met the selectivity acceptance criteria for NMP and NHP, respectively. For dilutional linearity 5 individual NHP samples were serially diluted from 1:10 to 1:320 and the % difference in sAXL-endogenous concentration was not exceeding ±12.6% of each other throughout all dilutions.

Conclusion
The data generated during the development demonstrated that the method is specific and selective to human sAXL. With acceptable linearity, accuracy and precision, the method was reliably used for the evaluation of the concentrations of sAXL in plasma from different human cancer types.