MECHANISTIC MODELING AND HEPATIC BIOMARKER DATA FROM GGF2 (CIMAGLERMIN ALFA)-TREATED SUBJECTS IN PHASE 1 CLINCIAL TRIALS SUGGEST LOW LIKELIHOOD OF PROGRESSIVE LIVER INJURY

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ABSTRACT

BACKGROUND: GGF2 (USAN cimaglermin alfa) is an investigational drug for the treatment of heart failure. During Phase 1 clinical trials, concomitant, transient elevations in serum aminotransferases (ALT/AST) and bilirubin meeting FDA DILI Guidance stopping criteria (Hy's Law) were observed in two treated subjects but resolved within 2 weeks, with no further LFT abnormalities over the following 1-3 years. GGF2 has no direct toxic effect on human hepatocytes in vitro, so further exploration of these cases was undertaken.

METHODS: Serial serum samples were assayed for novel hepatic safety biomarkers including mi-R122, full length keratin-18 (FL-K18), caspase-cleaved K18 (cc-K18), as well as traditional liver chemistries (AST, ALT, bilirubin). DILIsym® software, a mathematical, predictive model of drug-induced liver injury, was used to interpret the kinetics of these biomarkers. Liver enzyme profiles were used to back-estimate hepatocyte loss through apoptosis or necrosis.

RESULTS: In these two cases, mi-R122 values were elevated and consistent with liver origin of the ALT and AST elevations. The cc-K18/FL-K18 ratio supported apoptosis as the major mode of hepatocyte death. DILIsym estimated that the maximum loss of hepatocytes in the two subjects was 2-13%, which would not account for a significant rise in serum bilirubin. Therefore, a transient defect in bilirubin transport likely contributes more than hepatocyte death to the rise in serum bilirubin in these GGF2-treated subjects.

CONCLUSION: The two cases meeting the FDA DILI Guidance stopping criteria appear to be the result of a transient defect in bilirubin transport more so than hepatocyte death. They should therefore not be considered typical Hy's Law cases that would signal the potential for progressive liver failure.

INTRODUCTION

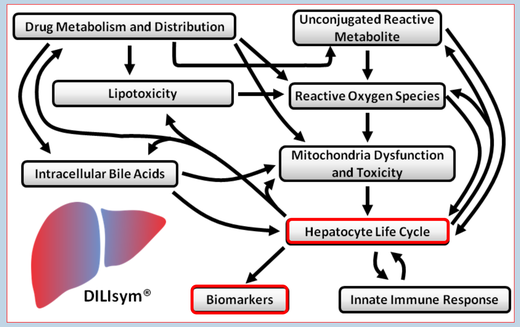
Cimaglermin alfa is a recombinant version of naturally occurring glial growth factor 2 (GGF2). Cimaglermin alfa acts at the cellular level on cardiomyocytes and neurons by interacting with ErbB3 and ErbB4 receptors. In Phase 1 clinical trials, dose-related improvements in cardiac function were observed in patients with heart failure following a single administration of cimaglermin alfa. However, clinical trials were suspended when 2 cimaglermin alfa-treated subjects experienced concomitant elevations in serum aminotransferases and bilirubin meeting the stopping criteria outlined in the FDA Guidance on Drug-Induced Liver Injury (DILI).[1]

Recently, the FDA identified the development and use of computational models as an important tool for assessing product safety.[2] Mechanistic, mathematical modeling can facilitate the interpretation of liver safety biomarker data by incorporating biomarker release and clearance kinetics and linking back the results to mechanisms underlying DILI. Previous work demonstrated the successful application of DILIsym, a mechanistic model of DILI, to aid in the interpretation of serum aminotransferase (but not bilirubin) elevations observed in healthy volunteers in a Phase 1 clinical study.[3]

In the current study, serial serum samples collected from Phase 1 clinical trial subjects were assayed for miR-122, FL-K18, and cc-K18. miR-122 is a liver-specific biomarker released during hepatocyte death. The ratio of cc-K18 to FL-K18 (the 'apoptotic index') has been proposed to estimate the relative contributions of necrosis and apoptosis to cell death. DILIsym software was used to interpret the kinetics of these biomarkers along with the traditional liver chemistries that had been measured in the trials.

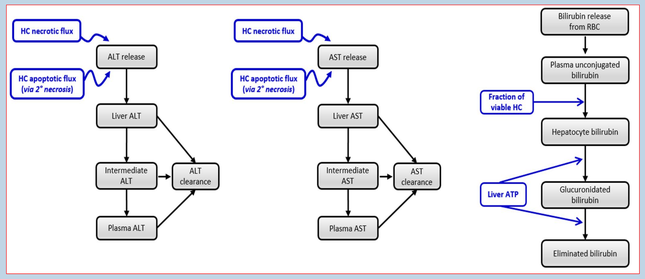
RESULTS

Overview of DILIsym



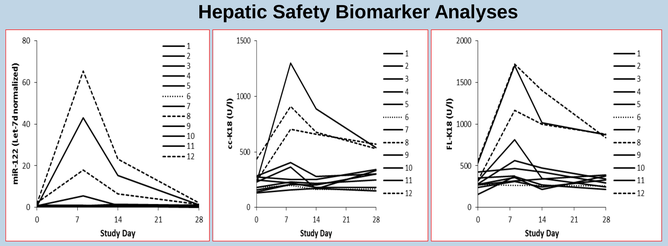
Schematic overview of the overall structure of DILIsym version 4B. Modules utilized for this application include the hepatocyte lifecycle and liver injury biomarker submodels. The hepatocellular mechanisms (drug exposure, hepatotoxicity mechanisms, adenosine triphosphate (ATP) depletion, etc.) leading up to cell loss were not considered herein. Instead, the method for simulating hepatocellular loss was an empirical, direct necrosis or direct apoptosis signal.

Biomarker Submodels in DILIsym



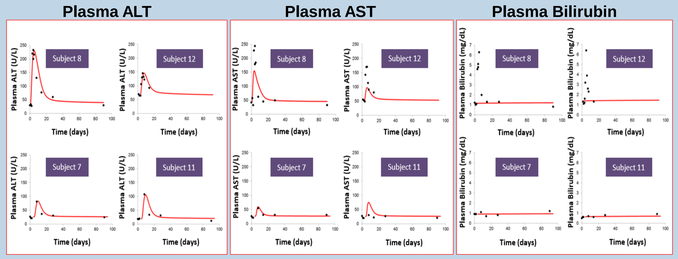
Diagrams of serum alanine transaminase (ALT), serum aspartate transaminase (AST), and bilirubin submodels within DILIsym version 4B.

Hepatic Safety Biomarker Analyses



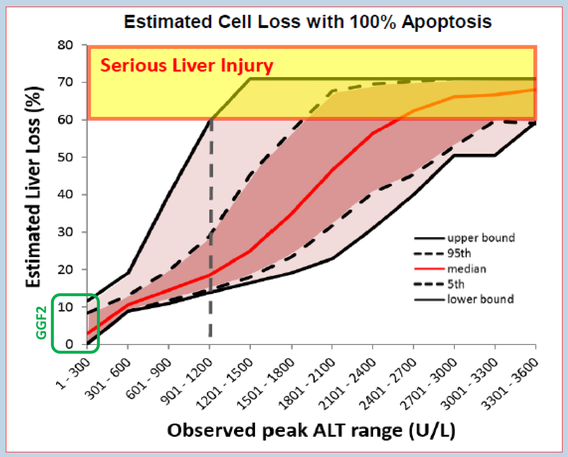
Serum levels of miR-122, cc-K18, and FL-K18 for 12 patients, including the 2 cases (Subjects 8 and 12, dashed lines), as well as others with elevated AST/ALT tests that fell short of meeting FDA DILI Guidance stopping criteria (solid lines), and one placebo-dosed individual (Subject 6, dotted line). In the 2 cases, mi-R122 values were elevated, consistent with liver origin of the ALT/AST elevations. Elevated levels of cc-K18 and FL-K18 were also observed in these subjects. Across all subjects with elevated cc-K18 and FL-K18, the 'apoptotic index' (ratio of cc-K18 to FL-K18) ranged from 0.53 to 0.76, suggesting that apoptosis was the major form of cell death.

DILIsym Baseline Human Simulation Results



Observed and simulated plasma levels of ALT, AST, and bilirubin for the 4 cimaglermin alfa subjects included in the DILIsym analyses. Closed black circles represent clinical data. Solid red lines represent simulated results due to 100% apoptosis. The 4 subjects included the 2 cases (Subjects 8 and 12), as well as 2 subjects with elevated AST/ALT tests that fell short of meeting FDA DILI Guidance stopping criteria (Subjects 7 and 11). The ALT model was optimized to the cimaglermin alfa ALT clinical data. AST and bilirubin data were not used for the optimization; plasma AST and bilirubin levels were simulated based on the amount of hepatocyte injury optimized to ALT dynamics. The lack of bilirubin elevations in the DILIsym simulations is not consistent with the marked rise in serum bilirubin observed in the 2 cimaglermin alfa subjects who met Hy's Law criteria.

Population-level Simulation Results



Using a simulated human population (SimPops) to account for inter-individual variation, DILIsym estimated that the maximum loss of hepatocytes in cimaglermin alfa-treated subjects was <13%. No bilirubin elevations were predicted for the range of estimated hepatocyte loss in cimaglermin alfa-treated subjects. ALT elevations in cimaglermin alfa-treated subjects are comparable to those observed with heparins, which do not cause clinically significant liver injury when taken as directed, and where hepatocyte loss is predicted to be <16% in healthy volunteers. "Serious Liver Injury" was defined here as >60% estimated hepatocyte loss, based on published reports of reductions in hepatocyte volume fraction >60% in patients in whom death was attributed solely to hepatic failure.[4] The SimPops analyses indicated that Serious Liver Injury is possible-likely when peak ALT >1200-1800 U/L.

METHODS

Hepatic Safety Biomarker Analyses Serum samples were analyzed for miR-122, FL-K18, and cc-K18. Analyses were performed on a total of 48 serum samples collected at 4 different time points relative to cimaglermin alfa (or placebo) administration from 12 subjects who participated in Phase 1 clinical trials. The subjects whose samples were analyzed include the 2 cases as well as others with elevated serum AST/ALT levels, and a single subject who was placebo-dosed. Traditional liver chemistries (AST, ALT, and bilirubin) were collected previously (i.e. in the clinical trials).

DILIsym Analyses DILIsym is a mechanistic, mathematical model of DILI (DILIsym Services Inc., http://www.dilisym.com), developed and maintained through the DILI-sim Initiative, a public-private partnership involving scientists in academia, industry, and the FDA. In the current study, DILIsym version 4B was utilized to estimate the amount of hepatocyte loss in cimaglermin alfa-treated subjects with elevated ALT/AST, and to simulate circulating bilirubin levels for the inferred amount of hepatocyte loss. DILIsym analyses were performed on the 4 clinical trial subjects with the highest elevations in serum aminotransferases, including the subjects meeting Hy's Law criteria.

DILIsym was used to approximate the ALT elevations observed in Phase 1 clinical studies following treatment with cimaglermin alfa by simulating dynamic changes in liver necrosis or apoptosis.

To account for the inter-individual variability in ALT response and hepatocyte regeneration from a given level of hepatocyte loss, a simulated human population (SimPops™) that consists of 300 healthy individuals was employed. In this SimPops, variability was introduced in the parameters relevant to ALT dynamics and hepatocyte regeneration. The range and variance of each parameter was obtained from the literature, and simulated hepatocyte loss and plasma ALT levels in the SimPops were validated using clinical data from the literature.

CONCLUSION

Mechanistic biomarkers and mathematical modeling were used to predict the maximum hepatocyte loss experienced by the two cimaglermin alfa-treated subjects. Although these subjects met Hy's Law Criteria the predicted extent of liver injury was not sufficient to account for the significant rise in serum bilirubin. Hence, these two subjects should not be considered typical Hy's Law cases. This study illustrates the capability of mechanistic liver biomarkers and mathematical modeling to improve liver safety risk assessment.

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