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Executive Director

Letter of support for drug-induced liver injury (DILI) biomarker

Summary

The Drug-Induced Liver Injury (DILI) work package 3 (WP3) of the SAFE-T consortium specifically aimed to address the current lack of sensitive and specific clinical tests to diagnose, predict and monitor drug-induced injury to the liver, which is a major hurdle in drug development.

The objectives of DILI WP3 were to qualify one or a set of new biomarkers with respect to:
- an early or earlier diagnosis of DILI as compared to current diagnostic rules
- the ability to predict DILI outcome, with particular emphasis on severe DILI/acute liver failure
- the prognosis and monitoring of progression and regression of DILI
- the differentiation between patients who incur true drug-induced liver injury from those who recover from the initial injury despite ongoing drug treatment (adaptors)

Originally, the overall strategy for biomarker selection was ambitious with regard to the initial selection, further exploration, and final confirmation within a variety of clinical trials.

However, given time constraints and the limited number of patients available by the end of 2014, the DILI-WP decided to investigate 16 new biomarkers selected largely from the first stage gate analysis in one subsequent analysis using all available datasets and to no longer separate an exploratory from a confirmatory phase. True confirmatory data which could support a Qualification Opinion are therefore currently not available. All results submitted now are considered exploratory in nature.

Scientific discussion

During the development, the applicant have conducted or evaluated (1) protocols that recruited patients diagnosed with DILI and (2) protocols that recruited patients without a diagnosis of DILI but who were on treatment with potentially hepatotoxic drugs and were prospectively monitored for several months. For all studies, cases with suspected DILI were ascertained by clinical judgment of the investigators and, subsequently, by the evaluation of an adjudication committee. All cases meeting the trial enrolment criteria were adjudicated, the great majority of those fulfilled the consensus criteria for

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DILI as published by Aithal et al. (1) (ALT ≥ 5xULN, OR ALP ≥ 2xULN; OR ALT ≥ 3xULN with simultaneous elevation in total bilirubin > 2xULN). The fact that none of the patients enrolled into the two prospective studies “protocol 4” and “protocol 5” developed DILI contributed to the purely exploratory nature of analyses of the data available. This is the obvious reason why no analysis could be presented with regard to the time-course of the increase in (conventional and novel) biomarkers, as the comparisons/analyses conducted (cases from “DILI-studies” compared to “liver healthy” subjects) were obviously not suitable to analyse the time-related course. As no DILI cases could be detected prospectively, any statements for potential contexts of use intended to be included in the Letter of Support have been derived from either (i) the comparison of the “DILI groups” patients with the data derived from healthy volunteers, or (ii) the comparison of different outcomes of DILI patients (liver failure vs. recovery).

The main statistical analysis method used for the identification of marker candidates is the calculation of receiver operating characteristics (ROC) for distinguishing the two outcome groups related to the corresponding context of use (CoU). More specifically, the estimated area under the ROC curve (AUROC) was taken as measure for discriminative power. This has been done using logistic regression for single predictor variables and single predictor variables with key covariates added in. The area under the ROC curve has also been calculated when classification trees have been fitted using many predictor variables and key covariates. From a methodological viewpoint, the applied methodology is considered suitable for the described purpose, i.e. to identify marker candidates for further research.

In addition to the studies mentioned above, the applicant have used data from the US-based drug-induced liver injury network (DILIN) network which was established to advance the understanding and research into DILI by initiating a prospective registry of patients with DILI. The DILIN provided 166 samples from patients with acute DILI of which 22 patients developed chronic DILI, and 16 underwent liver transplantation or died from liver-related complications. Moreover, raw data from a study published by Antoine D et al, (2) (‘Liverpool study’) were obtained and used to support performance of biomarkers for the specific setting of intrinsic DILI (acetaminophen (APAP)-induced DILI).

In the analyses conducted within the proposed Context of Use A (see below), which included the studies initiated by the applicant, the AUROC for none of the biomarker candidates performed similarly or almost similarly to the “conventional” biomarker ALT (where the AUROC was 0.99). This is partly attributable to the fact that ALT was the major inclusion criterion into the acute DILI studies and thus the benchmark against which the other parameters were compared, but also due to the restricted dataset available, including a limited number of observation times.

However, a decision was taken by SAFE-T to select a panel of altogether 8 candidate markers for further analysis, based on their relative performance in the overall analyses, and their ability to partially outperform the conventional biomarkers (especially ALT, but also total Bilirubin) in additional (subgroup) analyses. These analyses relate to the “sub classification” of DILI, i.e. the histological features hepatocyte necrosis, apoptosis, and immune activation.

The comparison of the observed severe DILI cases according to the currently used “Hy’s Law criteria” indicated that some of the biomarker candidates indeed have the potential to outperform especially the “conventional” biomarker ALT. From the analysis conducted, and the different time-points available for evaluation in the DILIN cases and the SAFE-T protocols, a preliminary conclusion was taken that at least one of the biomarkers has the potential to predict the outcome of DILI at an early time-point.

Moreover, the analysis of the cases of APAP induced DILI as reported by Antoine et al. (2) also showed generally better results for part of the selected biomarkers for the prediction of liver injury in patients with ingestion of an APAP overdose.

Overall, however, the development of such prediction rules based on biomarker data is still at a preliminary stage and will have to be verified in future studies conducted by sponsors to reduce the high burden of uncertainties and "degrees of freedom" that are inevitably still present in the current data set.

Proposed context of use:
Based on the results of the evaluations conducted, the applicant has proposed that CHMP issues a letter of support for the further development of the biomarkers, including the following context of use statements:

Context-of-use statement "A":
Based on preliminary data, the following biomarkers have potential as clinical DILI biomarkers that sponsors may choose to incorporate into their clinical trials to assess whether biomarkers provide additional information beyond the diagnostic value of ALT & TBIL according to the following mechanisms in the pathophysiology/pathogenesis (including the detection of severe DILI as defined by Hy's law criteria):
a. hepatocyte necrosis (CK-18, miR-122, total HMGB1, GLDH, SDH)
b. apoptosis (ccCK-18)
c. immune activation (hyperacetylated HMGB1, MCSFR1)

Context-of-use statement "B":
Based on preliminary data, the biomarkers hyperacetylated HMGB1, Osteopontin, Total Keratin 18 and MCSFR1 have potential as clinical DILI biomarkers that sponsors may choose to incorporate into their clinical trials to anticipate early a risk for progression of hepatocellular injury to severe DILI in patients in whom an initial DILI diagnosis has been established based on elevations of the standard marker ALT alone or in combination with TBIL.

Context-of-use statement "C":
Based on preliminary data, the following biomarkers: total HMGB1, total and caspase-cleaved keratin 18, miR-122 and GLDH have potential as clinical safety biomarkers that sponsors may choose to incorporate early (within the first 24 hours) in early stage clinical trials for the assessment of suspected intrinsic liver injury before ALT increases.

Comments on the development and recommendations regarding the proposed contexts of use:
The efforts undertaken by the applicant to generate data in order to develop biomarkers that could more reliably diagnose, predict the outcome, and classify Drug-Induced Liver Injury is acknowledged and welcomed. The applicant has suggested exploratory use of the biomarkers in further clinical development. However, the results presented are promising in parts and less promising in other parts and do not yet allow final conclusions with regard to potential utility in clinical practice. Their further validation and ultimately qualification will depend on appropriate future study designs (including the definition of the target population, definition of a success criterion with regard to clinical utility of the marker, rationale for sample size, methodology for internal and external validation of the statistical prediction model, adjustment for other covariates such as subject characteristics, time point of marker measurement, drug exposure) in which predictive/classification rules are established and validated in

order to assess the clinical utility of the marker (panel) for future patients. Clear plans for this further validation are, however, currently not available.

It is therefore concluded, that – despite acknowledging the overall need for the further development of these (and other potential biomarker candidates) for all 3 of the proposed contexts of use - currently a clear hierarchy of the three proposals appears obvious, and a recommendation for the further development and exploration of the biomarkers has to be graduated according to the results achieved, and the prospects of further research.

Recommendations

Recommendation 1:
Clear and unconditional support to encourage further research is given for the biomarker candidates included in the proposed Context-of-use statement B. It appears that the most promising results have been achieved within this context, and the direction for further research can be defined more easily:
The parameters *hyperacetylated HMGB1, Osteopontin, Total Keratin 18 and MCSFR1* have potential as clinical DILI biomarkers to identify and assess the risk of progression in patients with an established diagnosis of DILI based on current standard criteria. Sponsors may choose to incorporate these parameters into their clinical trials to anticipate early a risk for progression of hepatocellular injury to severe DILI in patients in whom an initial DILI diagnosis has been established based on elevations of the standard marker ALT alone or in combination with TBIL.

Recommendation 2:
Further promising results have been achieved for the biomarkers explored in the proposed context of use statement C, which also describes a field where the direction for further research can be more easily defined. However, it does not most obviously relate to the development of new chemical entities for the treatment of diseases, unless a similar “intrinsic” toxicity as for the well-established compound paracetamol (acetaminophen) will have been established for a new chemical entity.

The parameters *total HMGB1, total and caspase-cleaved keratin 18, miR-122 and GLDH* have the potential to be used as clinical safety biomarkers that sponsors may choose to incorporate in clinical trials with compounds having suspected intrinsic liver toxicity in order to potentially improve the early (within 24 hours) prediction of the occurrence of liver injury.

Recommendation 3:
For the proposed context of use statement A, the added value of the proposed biomarkers is less apparent, and the more promising results appear to be based on subgroup analyses exploring three defined types of liver injury, which in reality might be partly overlapping. Moreover, the proposed wording includes the highest level of uncertainty (“to assess whether biomarkers provide additional information”). As a consequence, the difficulties described for the future definition of research appear to be highest, and therefore, lowest priority for support is given for this context of use statement:
The following parameters have potential as clinical DILI biomarkers that sponsors may choose to incorporate into their clinical trials to assess whether they provide additional information beyond the diagnostic value of ALT and TBIL. Currently available data indicate that the potential for diagnostic value is related to the following mechanism of pathophysiology/pathogenesis of DILI:
a) CK18, miR-122, total HMGB1, GLDH, and SDH for hepatocyte necrosis
b) ccCK-18 for apoptosis
c) hyperacetylated HMGB1 and MCSFR1 for immune activation.

The presented results are considered exploratory in nature. For the time being, the proposed markers have to be considered marker candidates (albeit promising) for the potential contexts of use. Other investigated markers (e.g., FABP1, Cadherin 5 in context-of-use B) may help to improve the diagnostic performance.

Whereas further research is encouraged towards the prospective validation of the candidate biomarkers, the consortium is expected to make available/publish all data in a transparent and comprehensive way in order to facilitate future investigations and validation of these markers.

Any available pre-clinical data considered suitable to help with the prospective timely design of future investigations should similarly be made publicly available and be used for future research.

Sincerely,

Guido Rasi
Executive Director

1. Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, Hunt CM, et al. Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther 2011;89:806-815.
2. Antoine DJ, Dear JW, Lewis PS, Platt V, Coyle J, Masson M, Thanacoody RH, et al. Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital. Hepatology 2013;58:777-787.