Drug-Induced Liver Injury in Humans: The Case of Ximelagatran

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Drug-Induced Liver Injury in Humans: The Case of Ximelagatran

M. Keisu and T. B. Andersson

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Abstract Ximelagatran was the first orally available direct thrombin inhibitor under clinical development that also reached the market. Ximelagatran was tested in an extensive clinical programme. Short-term use (<12 days) in humans including the phase III clinical trials did not indicate any hepatotoxic potential. Increased hepatic enzyme levels were first observed at a higher frequency when evaluating the long-term (>35 days) use of ximelagatran (incidence of >3× upper limit of normal (ULN) plasma ALT was 7.9%). The frequency of elevated total bilirubin levels was similar in the ximelagatran and the comparator groups. However, the combination of ALT > 3×ULN and total bilirubin > 2×ULN was 0.5% among patients treated with ximelagatran and 0.1% among patients in the comparator group. Symptoms such as fever and rash potentially indicating hypersensitivity (immunologic type of reaction) were low and did not differ between ximelagatran and the comparators. The withdrawal of ximelagatran from the market and termination of the ximelagatran development program was triggered by safety data from a 35-day study, indicating that severe hepatic injury in a patient could develop after exposure to the drug has been completed and that regular liver function monitoring may not

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mitigate the possible risk of severe hepatic injury. As for many drugs causing liver injury, the standard preclinical toxicological studies provided no indication that ximelagatran affected hepatic functions. In addition, extensive investigations using human-based in vitro models have not been able to define mechanisms explaining the pattern of hepatic injury observed in long-term clinical trials. A pharmacogenomic study provided evidence that the ALT increases were associated with major histocompatibility complex (MHC) alleles DRB1'07 and DQA1*02 suggesting a possible immunogenic pathogenesis. This example provides important clues to the mechanism of idiosyncratic drug-induced liver toxicity.

Keywords Ximelagatran · Thrombin inhibitor · Pharmacogenetics · Transaminases · Bilirubin · In vitro liver toxicity models

1 The Clinical Picture

1.1 Background

New oral antithrombotic agents are under development with the aim to replace warfarin, a very effective anticoagulant but with serious side effects subject to slow onset of action, and an interaction profile including numerous foods and drugs. Patients on warfarin are also subject to frequent anticoagulation monitoring and dose adjustments (Hirsch et al. 2001; Ansell et al. 2001). The development of new antithrombotic agents has been facilitated by an understanding of the molecular basis of the interaction between coagulation factors and the nature of their substrates, mainly thrombin and factor Xa. The clinical benefit of any new such agent will depend critically on its safety and simplicity for patients to use.

Ximelagatran was the first orally available direct thrombin inhibitor under clinical development that also reached the market.

1.2 The Ximelagatran Development Program

Ximelagatran was tested in an extensive development program including the following indications/patient categories.

"Short-term treatment" (up to12 days; 35 days)

- Prevention of venous thromboembolic events after total hip and knee replacement; up to 12 days of treatment (also marketed) (Eriksson et al. 2003a,b; Francis et al. 2002, 2003).
- Extended prophylaxis of VTE following elective hip replacement and surgery for hip fracture; planned treatment length: 35 days (Agnelli et al. 2009).

"Long-term treatment"

- Stroke prevention in patients with atrial fibrillation (SPORTIF Executive Steering Committee for the SPORTIF V Investigators 2005; Executive Steering committee on behalf of the SPORIF III Investigators 2003).
- Secondary prevention of venous thromboembolism in patients with DVT/PE after initial (6 months) treatment with vitamin K antagonists; planned treatment length 18 months (Schulman et al. 2003).
- Treatment of deep vein thrombosis; planned treatment length 6 months (Fiessinger et al. 2005).
- Secondary prophylaxis after myocardial infarction; planned treatment length 6 months (Wallentin et al. 2003).

In the overall long-term population, 6,841 patients started ximelagatran treatment. The number of patients exposed for 3 months, 6 months, and 15 months were 6,581, 5,987, and 3,630, respectively. The median exposure time in the long-term trials was approximately 1 year. Longest exposure time exceeds 5 years. Data from patients treated for up to 5 years for stroke prevention with nonvalvular atrial fibrillation have been published (AstraZeneca Clinical Trials: http://www.astrazenecaclinicaltrials.com/article/527374.aspx).

1.2.1 Detection and Incidence of a Hepatic Signal

It is standard practice during clinical trials to monitor hepatic laboratory tests, such as serum aminotransferases (ALT; AST), total bilirubin (Bili), alkaline phosphatase (ALP), and to investigate their potential clinical significance as indicators of hepatotoxicity.

No evidence of hepatotoxicity was noted/observed with ximelagatran during routine preclinical assessment, or at the later extensive investigation of the potential mechanisms for the hepatic injury observed during human exposure (for a detailed description of the preclinical examinations, see later).

Short-term use in humans including the phase III clinical trials for prevention of VTE in elective hip or knee replacement and the experience from marketed use (approximately 12,000 to 15,000 patients) did not indicate any hepatotoxic potential/signal. In fact, at the end of the 7- to 12-day prophylaxis period in the combined studies, the incidence of ALT elevations $>3\times$ upper limit of normal (ULN) was small, e.g., 0.91% in the ximelagatran group and 0.70% in the warfarin group of patients who underwent total knee replacement. ALT level increases were consistently smaller in patients with ximelagatran than those treated with low-molecular weight heparin (the main comparator in the short-term orthopedic surgery studies conducted in Europe).

Increased hepatic enzyme levels were first observed at a higher frequency (4.3%) than in the control group in a study evaluating the long-term use of ximelagatran (i.e., planned treatment>35 days) in comparison with warfarin (Petersen et al. 2003). Following this observation, the frequency of hepatic laboratory

testing was increased in all long-term clinical trials; strict criteria for inclusion and exclusion of patients with raised hepatic enzymes were introduced, and patients who developed an increased hepatic enzyme value were investigated and followed according with a prespecified protocol and algorithm. The frequency of ALT elevations $>3\times$ ULN was 7.9% (6–13.2%) in the ximelagatran-treated group. In the majority of patients, the daily dose of ximelagatran was 36 mg $\times 2$, but doses from 24 mg $\times 2$ to 60 mg $\times 2$ have been tested. Overall, a dose relationship to the ALT elevations could not be established.

A frequency of ALT elevation $>3\times$ ULN of 0–2% was observed among patients treated with the comparators (placebo, warfarin, LMWH/warfarin, aspirin) (Lee et al. 2005). Among 233 patients treated with ximelagatran and 35 patients treated with placebo, the pattern of the hepatic enzyme elevations was evaluated. Of these, 76% (150) and 43% (15) were judged to have hepatocellular injury, while 24% (48) and 57% (20) were judged to have mixed or cholestatic injury (Lewis et al. 2008).

1.2.2 Other Clinical Features of the Hepatic Signal

Data from the 546 patients with ALT $>3\times$ ULN noted among the ximelagatran-exposed patients were analyzed to evaluate the time course of the first occurrence of an increase in the levels of ALT $>3\times$ ULN; 93% of the elevations occurred within the first 6 months and 98% within the first 12 months (Fig. 1). It was possible to document data on recovery in 96% of the 546 ximelagatran-treated patients with ALT $>3\times$ ULN. Subdividing recovery data according to whether patients still received or discontinued ximelagatran treatment could be demonstrated in 93% of the 342 patients who stopped treatment, while their ALT was elevated to $>3\times$ ULN, and in all 204 patients who continued treatment during the period of the ALT elevation (Fig. 2).

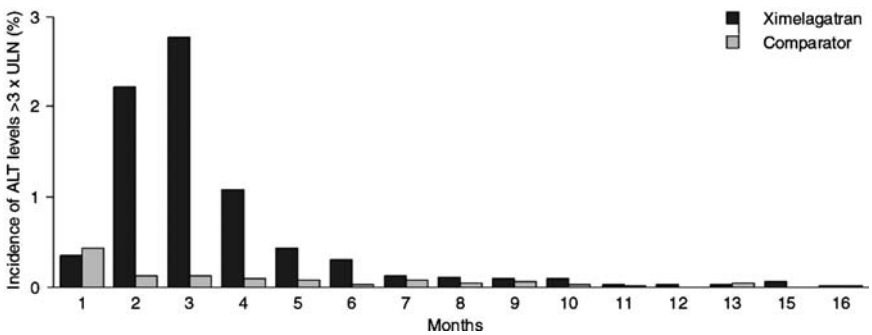

Fig. 1 Histogram of the number of patients with ALT levels of $>3\times$ ULN for the first time per month in each treatment group. *ALT* Alanine aminotransferase, *ULN* upper limit of normal (Lee et al. 2005).

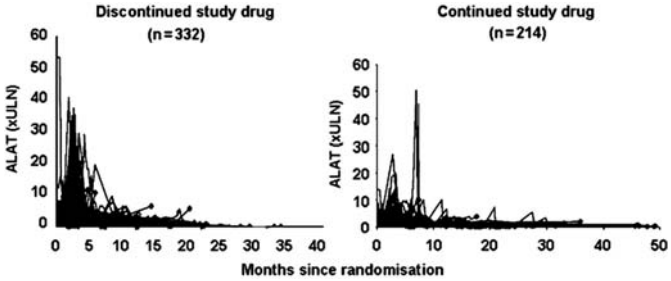

Fig. 2 ALAT elevations >3×ULN in patients treated with ximelagatran with and without discontinuation of study drug. Data from 546 out of 6,948 patients randomized to ximelagatran long-term (in studies SH-TPA-0002, SH-TPA-0003, SH-TPA-0004, SH-TPA-0005, SH-TPV-0003, SH-TPV-0002, SH-TPV-0005, SH-TPC-0001; see the AstraZeneca Clinical trials registry at http://www.astrazenecaclinicaltrials.com/). (Kindmark et al. 2008)

1.2.3 Mortality, Frequency of Bilirubin Increase and Symptoms Indicating Hepatic Disorder (Lee et al. 2005)

All-cause mortality in the total long-term treatment population was similar between the ximelagatran- and comparator-treated groups (3.9 vs 4.4%). The percentage of ximelagatran-treated patients with ALT >3× ULN who subsequently died did not differ compared with the percentage of those with ALT levels of >3× ULN who died in the comparator group (4.0 and 3.9%, respectively). The frequency of elevated total bilirubin levels was similar in both treatment groups: 4.1% in the ximelagatran-treated group and 3.6% in the comparator-treated group for bilirubin >1.5× ULN; the corresponding frequencies were 1.2 and 1.1% for bilirubin >2× ULN.

Concurrent elevations of ALT >3× ULN and total bilirubin>2× ULN under certain circumstances (in the absence of other explanations and with a hepatocellular pattern) is used as a potential indicator for more severe hepatic injury (FDA Clinical White Paper, November 2000, http://www.fda.gov/cder/livertox/clinical.pdf). In the long-term ximelagatran trial program *regardless of etiology*, the frequency of patients with the combination of ALT >3× ULN and total bilirubin >2× ULN was 0.5% among patients treated with ximelagatran and 0.1% among patients in the comparator group.

An analysis in the ximelagatran clinical trial program showed no difference in the number of adverse events "possibly associated with a hepatic disorder" (abdominal pain, nausea, fatigue, jaundice) between the two groups. Such symptoms were noted among 20.2% of the ximelagatran-treated patients and 19.2% of the comparator-treated patients. As expected, in both the ximelagatran and the comparator groups, patients with ALT levels of >3× ULN had a higher incidence of adverse events "possibly associated with a hepatic disorder" than those without ALT level elevations. Symptoms such as fever and rash potentially indicating hypersensitivity (immunologic type of reaction) were low (rash: ximelagatran/comparator: 4.3/4.7%; fever: ximelagatran/comparator: 1.7% in both groups) and did not differ between ximelagatran and the comparators (data on file; AstraZeneca).

1.2.4 Reintroduction

Overall, 18 patients who had an ALT level of $>3\times$ULN and discontinued ximelagatran treatment temporarily subsequently resumed treatment. The duration of reexposure was >2 months in 16 of the 18 patients, and ALT was checked at least monthly during reintroduction. Seventeen patients had no further ALT elevation. One patient developed an ALT elevation of $>3\times$ ULN 65 days after reintroduction of ximelagatran treatment without any signs or symptoms of hypersensitivity; after discontinuation of ximelagatran treatment, the patient's ALT level returned to normal (Lee et al. 2005).

1.2.5 "Extended Prophylaxis" (35 Days) Following Elective Hip Replacement or Surgery for Hip Fracture

When 1,158 patients had been randomized and 641 patients had completed the treatment period in this study, it was prematurely stopped due to the increased liver enzymes.

Overall, 58 patients (31 treated with enoxaparin and 27 treated with ximelagatran) had developed ALT $>3\times$ ULN. Eleven of the ximelagatran-treated patients showed an ALT increase after the end of the study treatment, all detected at the routine safety screening on day 56 (i.e., 3 weeks after the end of drug treatment). Three patients also showed symptoms of liver injury (nausea, fatigue, weight loss). One of these patients developed an ALT elevation of up to $46\times$ULN, bilirubin elevation of $17\times$ ULN and INR of 1.8. She received vitamin K and no other specific treatment.

All patients with ALT elevations, including the patients with symptoms, recovered (Agnelli et al. 2009).

The withdrawal of ximelagatran from the market and termination of the ximelagatran development program was triggered by the new safety data from the 35-day study described above, indicating that severe hepatic injury in a patient could develop after exposure to the drug has been completed, and that regular liver function monitoring may not mitigate the possible risk of severe hepatic injury.

2 The Pharmacogenetic Study (EXGEN)

The higher incidence of ALT cases in the ximelagatran-treated patients in northern Europe compared with Asia was a signal that there may be an underlying genetic factor. A retrospective case-control pharmacogenetic study was conducted based on 74 cases and 130 matched controls and included both a genome-wide tag single nucleotide polymorphism (SNP) and a large-scale candidate gene analysis (EXGEN study) (Kindmark et al. 2008). The results indicated a genetic association between patients carrying the major histocompatibility complex (MHC) class II

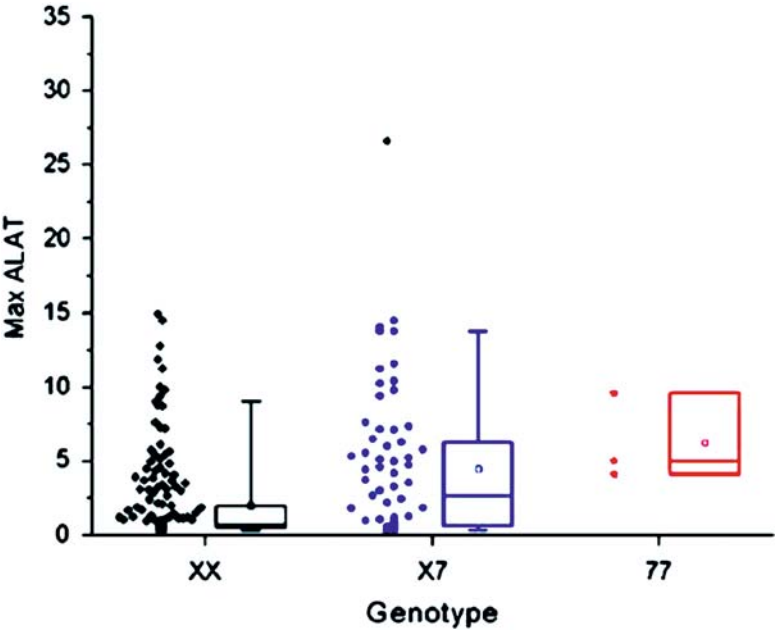

Fig. 3 DRB1*0701 genotypes versus maximum ALAT (in units of xULN) in all samples from EXGEN. Subjects designated as *XX* carry two alleles of DRB1 that are not DRB1*0701, as *X7* are heterozygous for DRB1*0701, and as *77* are homozygous for DRB1*0701. The scatter plots show the maximum ALAT in each genotype group, demonstrating the overlap in ALAT measurements between individuals in different genotype groups. The box-whisker plots (in which the box represents the 25th–75th percentiles of the distribution, the *whiskers* extend to 5th and 95th percentiles, the *central line* the median and the *small square* the mean) demonstrate that the means and distributions of maximum ALAT clearly differ between the genotype groups, with a trend towards increased maximum ALAT with increased copy number of DRB1*0701. (Kindmark et al. 2008).

alleles DRB1*07 and DQA1*02 and ALT increases during ximelagatran treatment (Fig. 3). This result was replicated in a restricted number of individuals, 10 cases and 16 controls. SNP analysis was done for the 20 top-ranked genes, and both DRB1*07 and DQA1*02 showed significant evidence for replication. Interestingly enough, the DRB1*0701 has a broadly similar geographic distribution to the ALT observations with a carrier frequency of approximately 11% in Scandinavia versus 0.3% in Japan (Berlin et al. 1997; Gibert and Sanchez-Mazas 2003). The results suggest that ximelagatran may induce an immunological response resulting in liver injury. However, the clinical picture does not suggest a classical immunological response because patients who experienced an increased ALT did not show evidence of fever, rash, or eosinophilia. The preclinical studies did not provide evidence for a possible immunological response upon exposure to ximelagatran. However, allergenicity studies had shown that guinea pigs could be sensitized by subcutaneously administered ximelagatran, and skin reactions were reported in 7 of approximately 20 individuals exposed to ximelagatran during manufacturing.

After the discovery of the genetic association of elevated ALT with genes involved in the immune system, a retrospective study was initiated to investigate the possible presence of drug-specific immune cells. Despite the fact that patients included in the earlier clinical trials might not have been exposed to the drug for over 4 years, 2 of 21 patients did demonstrate a weak positive lymphocyte transformation test (LTT). Both of these patients had ALAT $>4\times$ULN and one was heterozygous for DRB1*0701.

Drug-induced adaptive responses can arise because the drugs are metabolized into reactive metabolites that bind to proteins, which act as neoantigens (the hapten hypothesis). However, there is no evidence from the preclinical investigative program for the formation of reactive metabolites which could support such a mechanism. Drugs may also activate an immune response by forming a low-affinity association directly with the MHC molecules (Pichler 2002). Using a proprietary competitive binding assay (De Groot 2006), the company EpiVax (Providence, US) provided evidence that ximelagatran and its intermediate metabolite, melagatran-ethyl, at high concentrations, were able to inhibit the binding of peptides to HLA-DRB1*0701 and not to some other common DRB1 protein alleles (Kindmark et al. 2008). A low affinity binding is likely to result in an immune reaction restricted to a T cell but not a B cell response, which could be consistent with the apparent absence of classical signs of hypersensitivity in patients treated with ximelagatran. The sensitivity and specificity of the DRB*07 test in the EXGEN study were 47 and 83%, respectively, which indicates that other factors than HLA-DR1*07 contribute to the observed liver injury.

3 Preclinical Toxicological Studies

3.1 Summary of the Standard Preclinical Toxicological Studies

During the development programme, routine preclinical and toxicological studies showed no indications of any hepatic effects of ximelagatran in any species studied. After the detection of ALT elevations in the first clinical long-term treatment studies, further animal studies were initiated. In these studies, rats, dogs, and cynomolgus monkeys were exposed to high doses of ximelagatran, and blood sampling was performed more frequently than in previous studies. The results were negative: no ALT elevations related to ximelagatran treatment were observed.

3.2 Extended Mechanistic Studies

An extensive effort to investigate whether ximelagatran perturbed cellular functions that possibly could be involved in liver injury was initiated when the first indication of ALT elevations in the clinical study program appeared (Kenne et al.

2008). A very broad approach was adopted since the preclinical studies did not indicate any toxicity that could be related to liver injury, and the clinical study program at the time when the mechanistic studies were planned did not indicate any specific mechanism. Later the pharmacogenetic investigation observed a strong genetic association between patients who experienced ALT elevations and MHC alleles suggesting a possible immunological response.

Many different mechanisms can contribute to DILI (Lee 2003; Navarro and Senior 2006; Park et al. 2005). One of the most common causes of hepatotoxicity is the cytochrome P450 (CYP)-dependent formation of reactive metabolites that are either directly hepatotoxic or form adducts with hepatic proteins, potentially triggering an immune response. Other potential mechanisms of DILI include disruption of mitochondrial functions, inhibition of drug metabolism pathways, and inhibition of bile acid transport (Navarro and Senior 2006). Orphan receptors regulating drug-metabolizing enzymes and several vital functions in the cell, such as cholesterol and bile acid homeostasis, may be targets for hepatotoxic compounds (Repa and Mangelsdorf 2000; Wang and LeCluyse 2003). After oral administration, ximelagatran is rapidly absorbed and bioconverted to the active form, melagatran, in a two-step process involving ester cleavage and reduction of the amidoxime group (Eriksson et al. 2003c). None of the major human CYP isoenzymes appear to be involved in either of these steps (Bredberg et al., 2003; Clement and Lopian 2003). Ximelagatran, melagatran, and the intermediate metabolites, ethyl-melagatran and hydroxy (OH)-melagatran, have also been shown not to inhibit CYP isoenzymes in vitro (Bredberg et al. 2003). Ximelagatran has a predictable and reproducible pharmacokinetic/pharmacodynamic profile, with low inter- and intraindividual variability (Wolzt et al. 2005). In patients receiving oral ximelagatran 36 mg twice daily, the mean peak plasma concentration (C_{max}) was 0.3 μM for ximelagatran and 0.5 μM for melagatran (Wolzt et al. 2003). Concentrations of ethyl-melagatran and OH-melagatran were consistently below 0.1 μM. The concentrations of ximelagatran and its metabolites have not been determined in human liver tissue in vivo. Experimental systems were selected to investigate the potential effects of ximelagatran at the cellular, subcellular, and molecular level. Cell systems used included fresh and cryopreserved human hepatocytes, as well as human-derived hepatoma cell lines (HepG2 and HuH-7). A limitation with all these systems is that, for technical reasons, only short-term exposure can be studied. However, drugs with known clinical hepatotoxicity generally show cytotoxic effects during short-term exposure, even if clinical signs and symptoms only develop after several months of treatment (Gomez-Lechon et al. 2003).

The studies performed determined the effects of ximelagatran and its metabolites across a broad range of concentrations (up to 300 μM) in order to include higher concentrations than those found in plasma at therapeutic dosing (Table 1).

An alternative mechanism for transient increases of serum AST and ALT in patients treated with fibrates has been suggested to involve induction of gene transcription (Edgar et al. 1998). However, ximelagatran (1–30 μM) did not induce transcriptional activation of the human ALT1, ALT2, or AST1 promoter constructs in either HuH-7 or HepG2 cells.

Table 1 Summary of results from the mechanistic in vitro studies

Function tested	Results
Cell viability	Human hepatocytes tolerate ximelagatran well up to at least 200 μM (ATP content)
Apoptosis	An effect was first observed after exposure of HepG2 cells at 100 μM ximelagatran for 24 h
Calcium homeostasis	No effects on calcium homeostasis were observed after 15 min exposure of HepG2 cells up to 300 μM of ximelagatran
Mitochondrial functions	No effects in isolated mitochondrial preparations on: state 4 respiration, ADP-stimulated or 2,4-dinitrophenol-stimulated respiration, the respiratory control ratio, the adenosine diphosphate/oxygen ratio, the rate of calcium-induced mitochondrial swelling at 50 μM. Loss of MMP was seen only at the highest concentration of ximelagatran tested, 300 μM, in mitochondria exposed for 24 h. No effects on β-oxidation of fatty acids up to 300 μM
Reactive oxygen species (ROS)	No stimulation of intracellular ROS in primary human hepatocytes up to 100 μM
Inhibition of biliary transporter proteins	Neither a substrate nor an inhibitor of human bile salt export protein (BSEP)
Reactive metabolites	No indication for a reactive metabolite pathway. No glutathione adducts identified. No indication of protein adducts in human hepatocytes or subcellular fractions at concentrations up to 300 μM
Nuclear receptors	No indication of activation of human PXR and PPARα nuclear receptors or activation of TRβ, LXRα, LXRβ, and FXR coactivator recruitment

4 Concluding Remarks

As for many drugs causing liver injury, the standard preclinical toxicological studies provided no indication that ximelagatran affected hepatic functions. In addition, extensive investigations using human-based in vitro models have not been able to define mechanisms explaining the pattern of hepatic injury observed in long-term clinical trials. ALT elevations, mostly asymptomatic, transient, and occurring during the first 6 months of treatment, were observed in 7.9% of the patients treated with ximelagatran in the long-term (>35 days) clinical trial program. Elevated ALT levels were also noted in some patients 3 weeks after the end of drug treatment in a 35-day study. A few patients developed severe and symptomatic hepatic injury; in one patient, the injury developed weeks after the end of 35 days of ximelagatran exposure. Despite extensive analyses, we found no way to predict which patients are prone to develop severe hepatic injury or to devise a monitoring scheme that would allow early identification and withdrawal of patients from treatment before they developed severe hepatic injury.

The pharmacogenomic study provided evidence that the ALAT increases were associated with MHC DRB1'07 and DQA1*02 suggesting a possible immunogenic pathogenesis. However, further regulatory studies would have been needed before this test could have been implemented as a screening tool in the clinic. Genetic

associations have, however, the potential to be important risk mitigation components in future drug development programs.

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