



Note - presentation notes may be read by double clicking on the icon to the left

*FDA/CDER, PhRMA, AASLD:  
Recent Research Advances in Drug-Induced Liver Injury 2009-04-08*

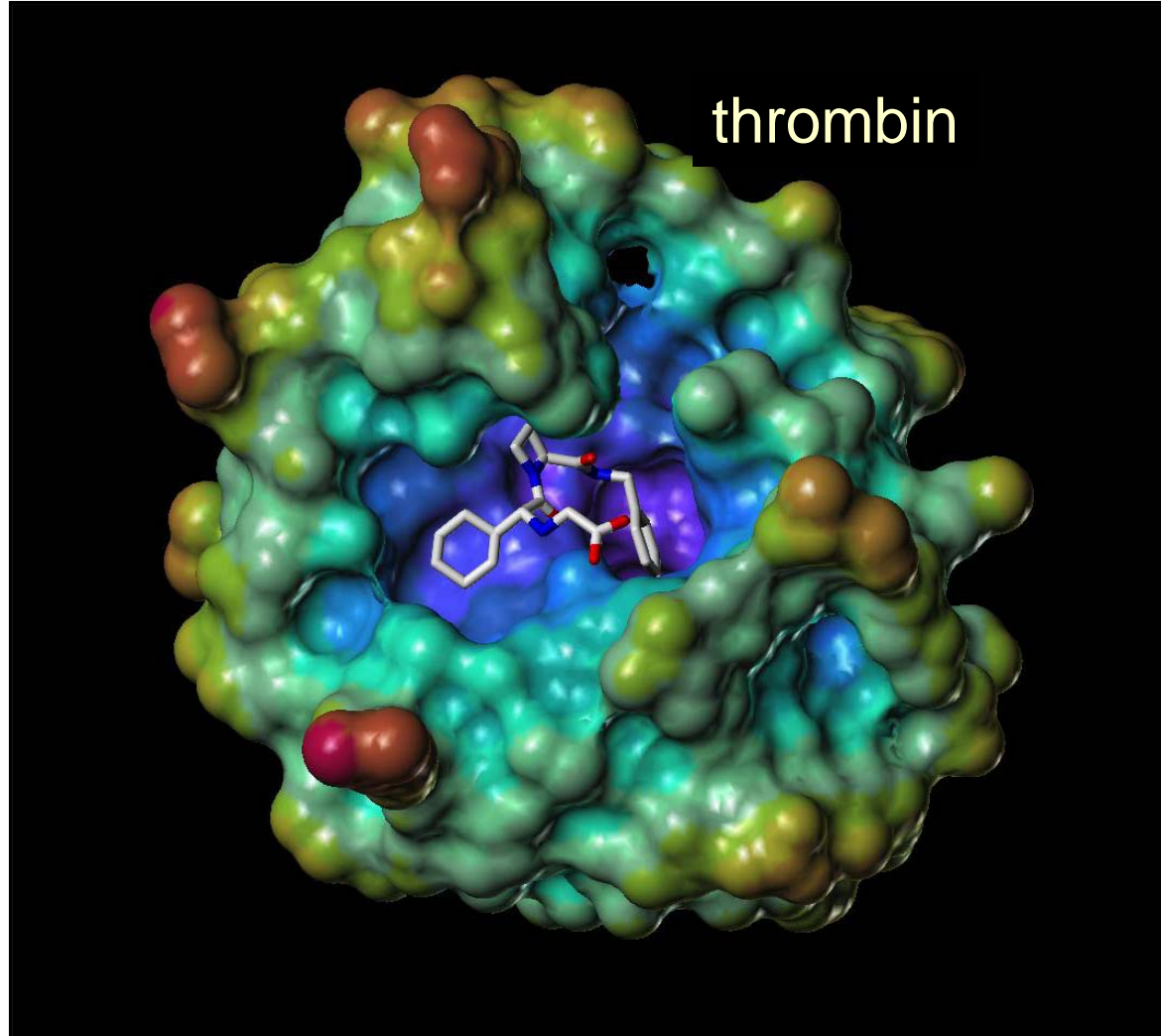
# Systems biology approach to ximelagatran injury

*Karin Cederbrant, AstraZeneca R&D.  
Safety Assessment/Immunotoxicology*

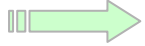


# Clinical background to ximelagatran and elevated ALAT levels

# EXANTA™ (ximelagatran) was developed as an oral direct thrombin inhibitor



Ximelagatran



Melagatran  
(active drug)

Mw ~470 g/mol

Binds rapidly,  
reversibly and  
competitively to  
active site

$K_i=0.002\mu\text{M}$



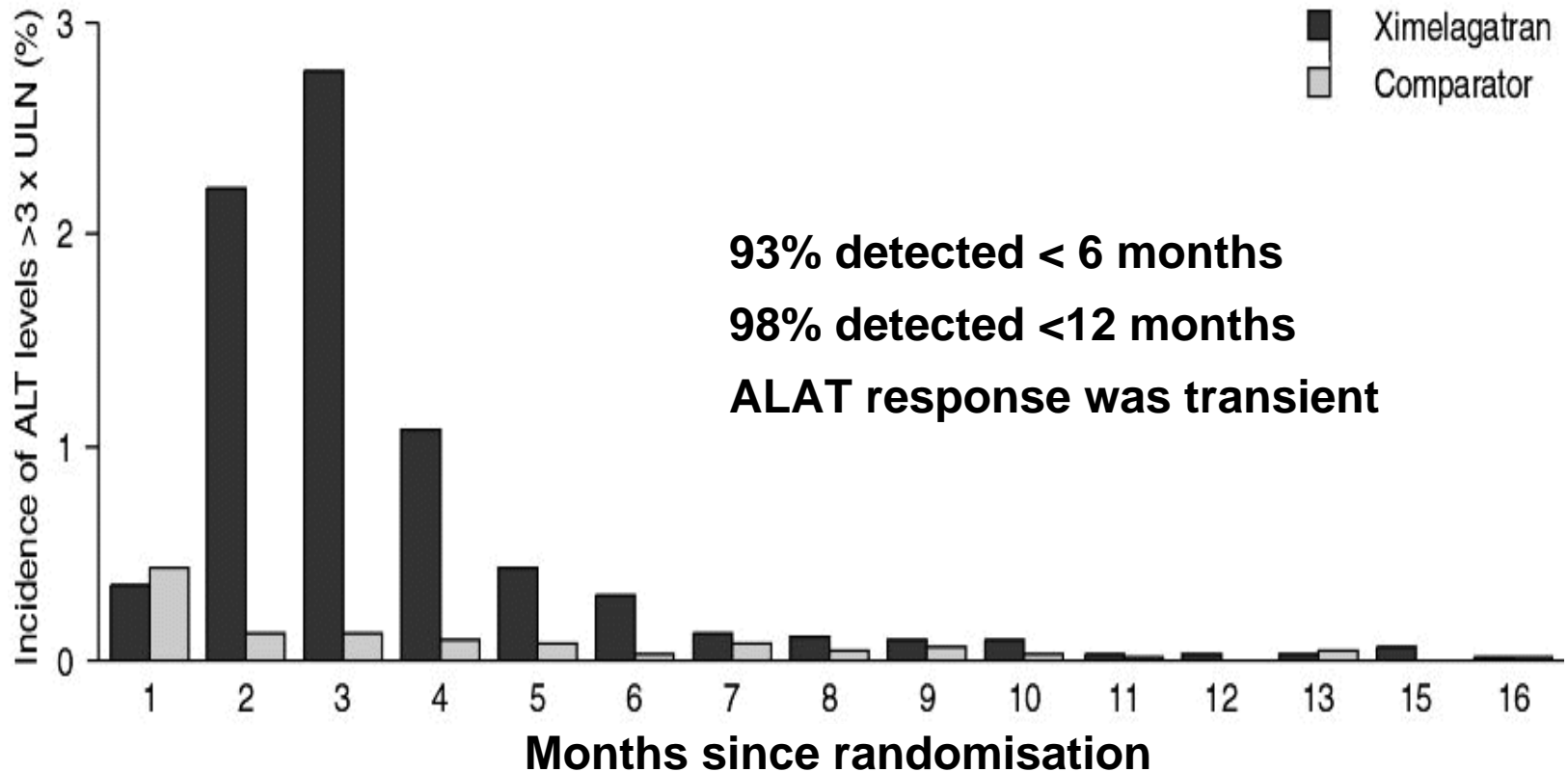
# Incidence of Hepatic Tests Elevations

## Long-term Studies (>35 Days)

<b>Parameter</b>	<b>Exanta n=6948</b>	<b>Comparator n=6230</b>
<b>ALAT &gt;3 xULN</b>	<b>546 (7.9%)</b>	<b>75 (1.2%)</b>
<b>ASAT &gt;3 xULN</b>	<b>354 (5.1%)</b>	<b>50 (0.8%)</b>
<b>Bilirubin &gt;2 xULN</b>	<b>86 (1.2%)</b>	<b>66 (1.1%)</b>
<b>ALP &gt;3 xULN</b>	<b>47 (0.7%)</b>	<b>22 (0.4%)</b>



# Pattern of serum ALAT (>3xULN) observed with ximelagatran treatment >35 days.





# Short term treatment

- No evidence of hepatotoxicity in short-term treatment

## The EXTEND study

- Ximelagatran in hip replacement and hip fracture surgery; 35 days treatment
  - Approx 386 patients treated for 35 days and passed the day 56 assessment
    - *One patient developed severe symptomatic liver disease after having normal ALAT value at day 35. Reaction started on day 62.*
    - *11 patients developed ALAT > 3xULN between day 35 and day 56*

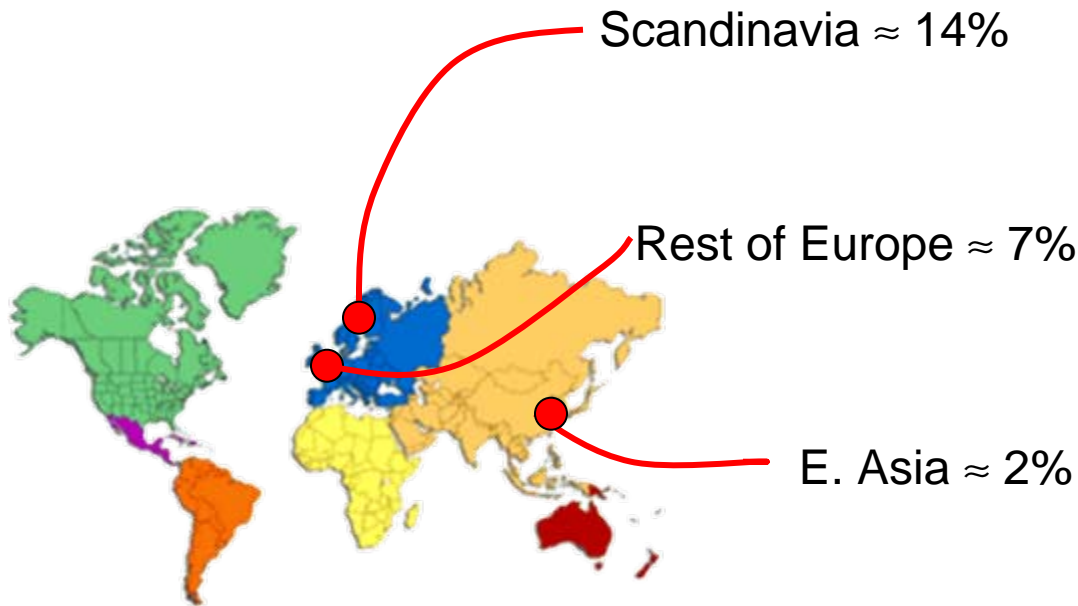
EXANTA™ withdrawn from clinical development in Feb 2006



# The EXGEN pharmacogenetic case-control study

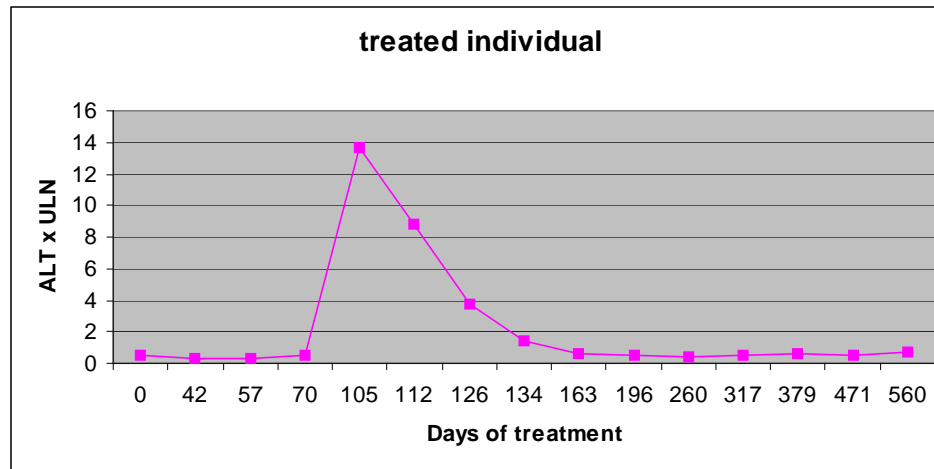


The geographic differences in ALAT elevation (>3xULN) indicated an underlying genetic predisposition



Increasing gradient:  
South→North  
East→West

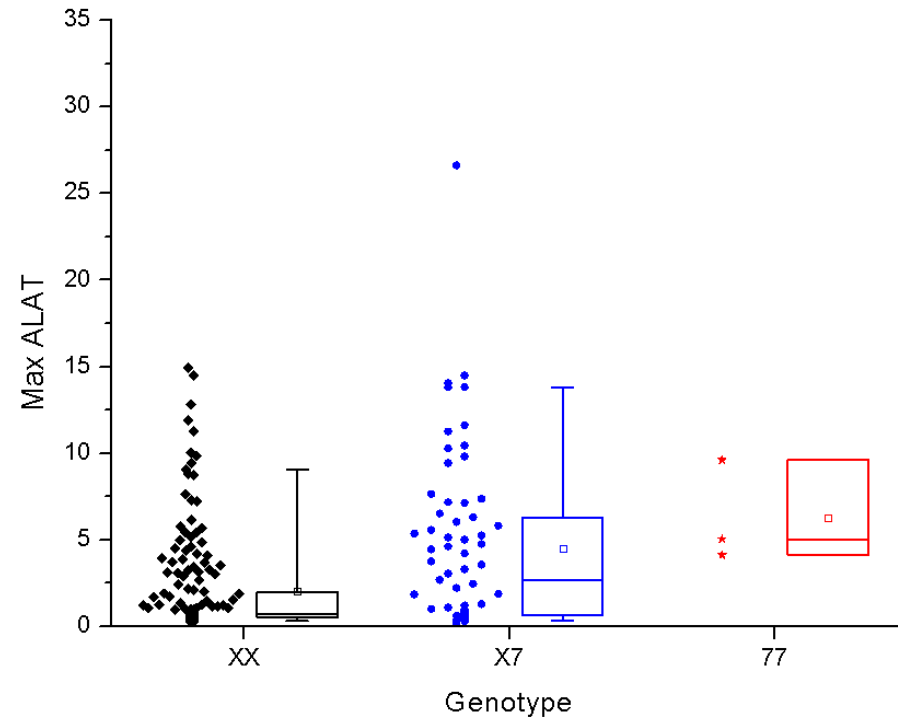
# Selection of cases and controls



- **78 cases**
  - ALAT elevation of  $\geq 3xULN$  and typical pattern
- **131 matched controls**
  - Normal ALAT during treatment ( $\leq 1xULN$ )
- **39 intermediate controls**
  - ALAT elevation between  $1xULN$  and  $3xULN$

# Results: HLA-association with ALAT elevations

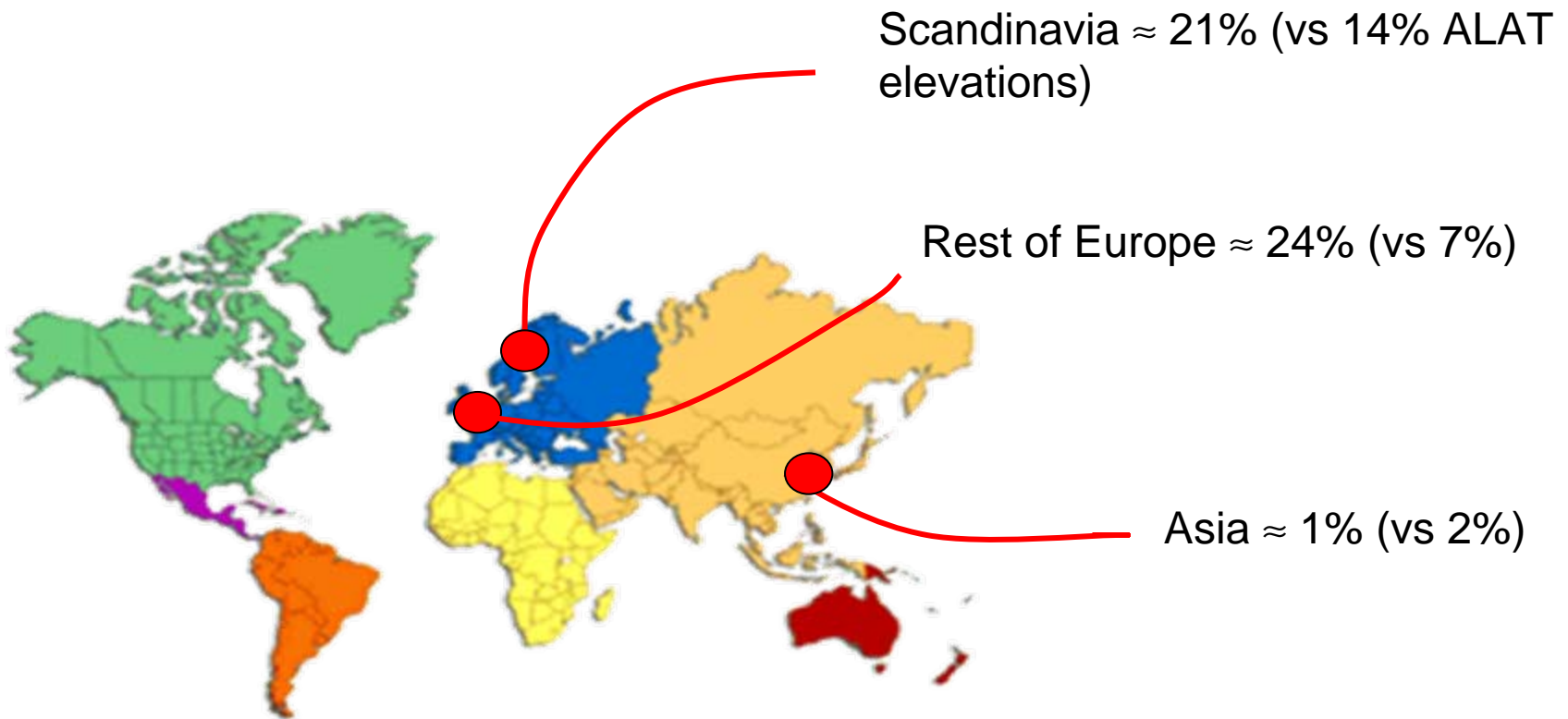
- Highest-ranked SNP close to *HLA-DRB1* ( $p=10^{-7}$ )
- The functional allele responsible for this association was DRB1\*0701 ( $p=10^{-6}$ )
- DRB1\*0701 was found in nearly half of the observed cases:
  - Sensitivity = 47%
  - Specificity = 83%



HLA DRB1\*0701 genotype vs maximum ALAT. Levels of ALAT in all subjects with 0, 1 and 2 copies of the allele. *Kindmark et al 2007.*



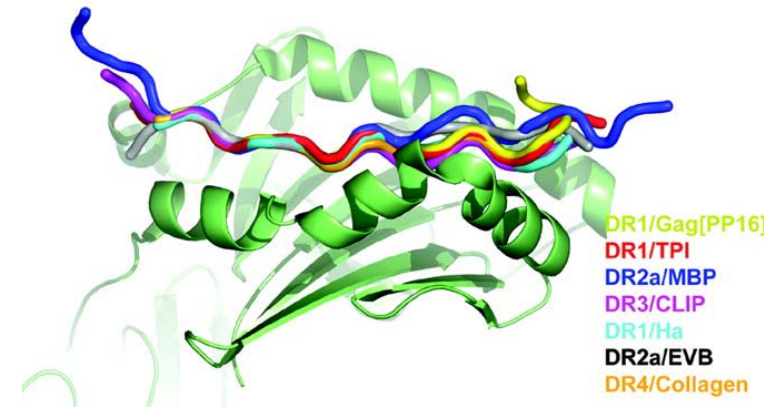
# Geographical distribution of DR7 is similar to ALAT elevations





# Characteristics of the HLA-DRB1\*0701 allele and gene product

- DRB1\*0701 forms extended haplotype with DQA1\*02 ( $p=10^{-5}$ )
- DRB1\*0701-DQA1\*02 associated with elevated ALAT:
  - Autoimmune hepatitis type 2
  - Hepatotoxicity during antituberculosis treatment



Role of HLA-DR is to present antigens to T-cells (eg. drug-modified peptides)

*Zavala-Ruiz et al (2004)*



# Ximelagatran Proteomics/Metabonomics study



# Ximelagatran Proteomics / Metabolomics Study

- **Retrospective case-control study on stored plasma samples from clinical trial Sportif III**
- AZ Safety Assessment Södertälje/Molecular Toxicology in collaboration with BG Medicine (Waltham, USA)

## Objectives:

- **Biomarkers** indicative of **pre-disposition** to developing ALAT elevation. 27 cases and 58 controls.
- **Biomarkers** of putative **treatment effect** in subjects who developed ALAT elevation. Data from 13 cases with both pre-dose and pre-ALAT peak or at ALAT peak sample.
- Putative biological **mechanism** for ALAT elevation



# Platform Overview

- Analytical platforms

- Polar LC/MS (at AZ)
- NMR (at AZ)
- Lipid LC/MS (at BGM)
- GC/MS (at BGM)
- Polar LC/MS (at BGM)
- Proteomics (at BGM)

- Data analysis (both at BGM and AZ)

- Approx 1200 analytes were used for statistical analysis



# Nominated Hypotheses

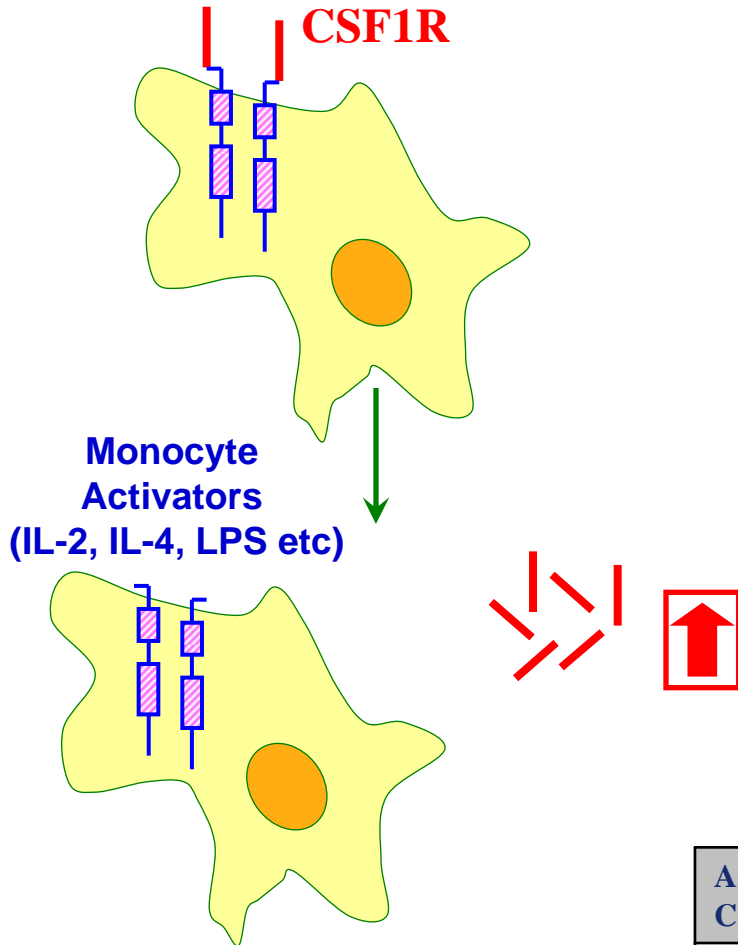
Validation ongoing

- Predisposition – Pyruvate hypothesis
- Predisposed patients have low levels of plasma pyruvate (e.g., low carbohydrate diets) and ximelagatran treatment promotes a further decrease in plasma pyruvate:
  - May result in reduced hepatocyte capacity to deal with oxidative stress, cells may become sensitised to ximelagatran.
- Treatment effects
  - Immune mechanism: Monocyte colony stimulating factor 1 receptor (CSF1R) shedding

From an academic perspective several man years of analysis could be usefully applied to this data set !

# Immune mechanism hypothesis

## CSF1R Ectodomain Shedding and Monocyte Activation



Presence of soluble CSF1R in serum - indication of monocyte activation.

Increased monocyte activation may potentiate antigen presentation to T-lymphocytes.

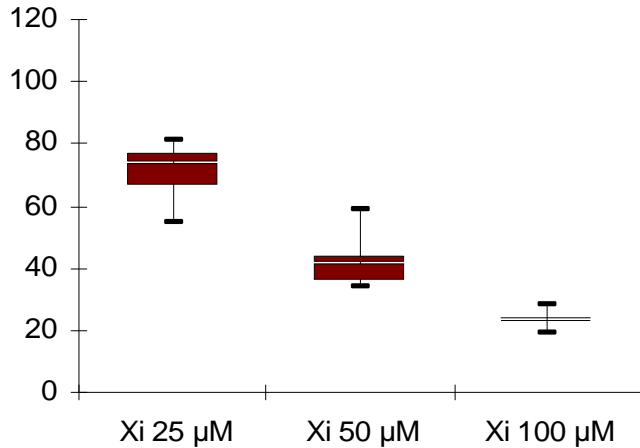
If ximelagatran has the ability to act as an immunopotentiator this might facilitate generation of drug-antigen specific lymphocytes.

Analyte Code	Identification	MFC	%Change	Raw p (W)	FDR p (W)
63152	CSF1R	1.31	41.55	0.004	0.097

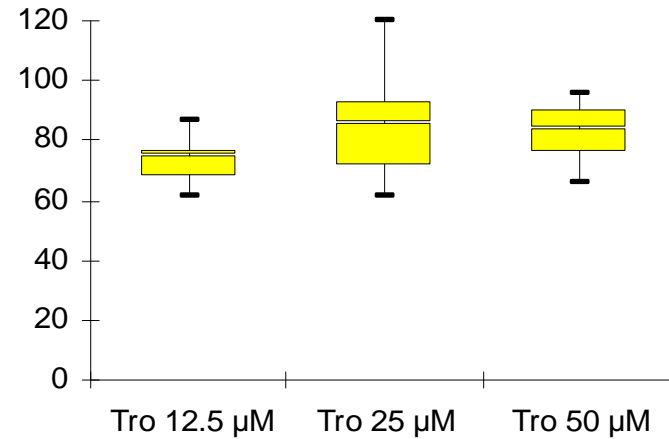


# In vitro verification study #1: Human primary monocytes (n=5). Flow cytometry analysis.

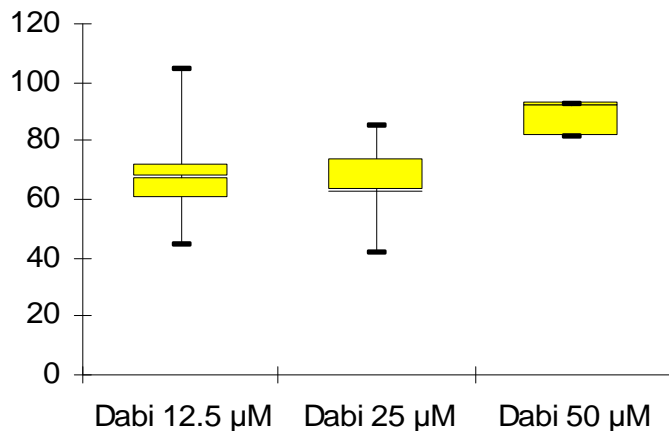
### CSF1 R, 20 h with Ximelagatran



### CSF1R, 20 h with Troglitazone



### CSF1R, 20 h with Dabigatran

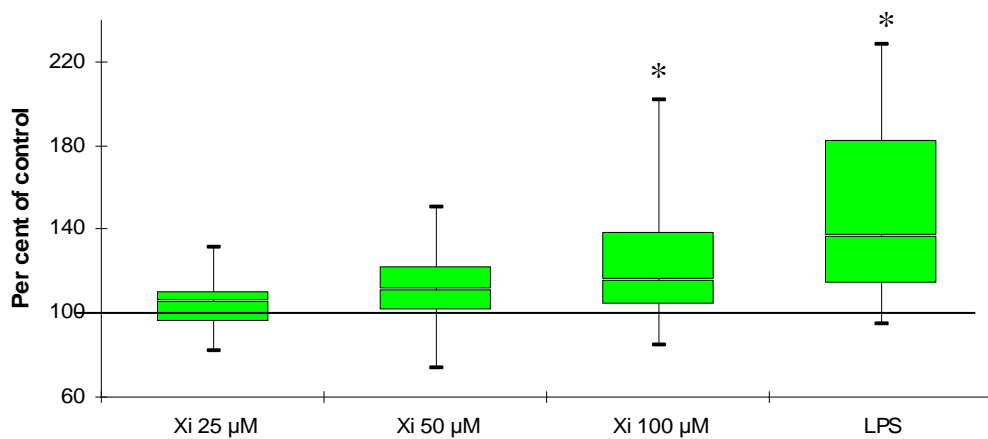


- Shedding of CSF1R showed a dose-response relation after exposure to Ximelagatran but not to Troglitazone and Dabigatran *in vitro*

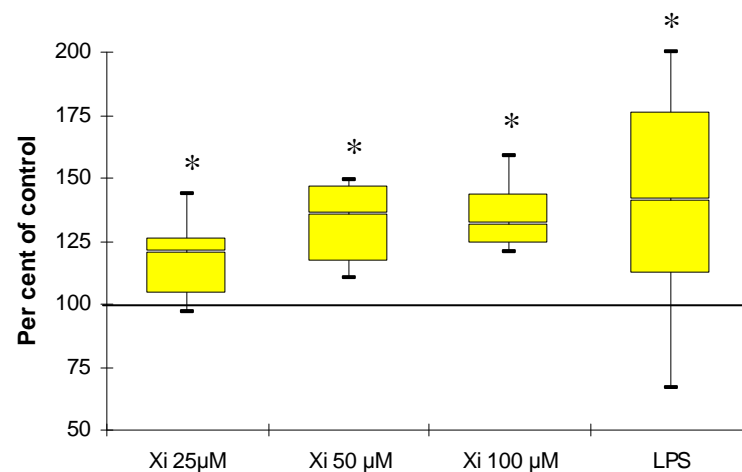


## In vitro verification study #2: Human primary monocytes (n=10). Flow cytometry analysis.

HLA-DR 20 hours



CD14 20 hours



- Ximelagatran also induced a dose-related significant increase of the common activation markers HLA-DR (NB results from Exgen-study) and CD14.



# Exanta

## Possible Immune mechanism



# Antigen-specific T-cell reactions in the liver

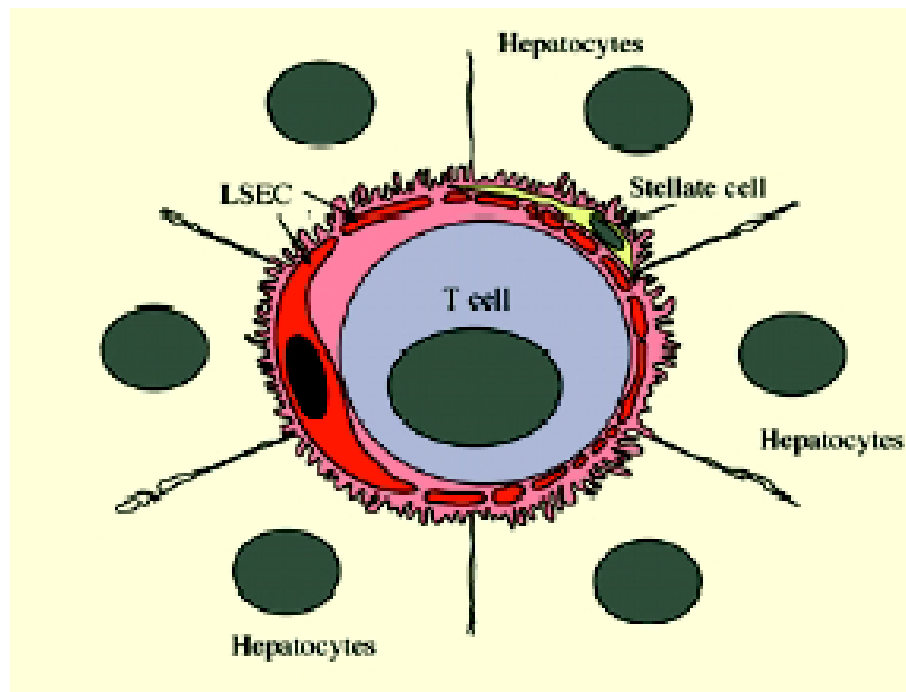


Fig. 1. Microanatomy of the liver sinusoid. Schematic drawing of the microarchitecture of the liver showing the relative positions of hepatocytes, Kupffer cells, sinusoidal endothelial cells (LSEC), stellate cells and liver-associated lymphocytes.

Knolle and Gerken -2000



# Diagnostic value of specific T cell reactivity to drugs in 95 cases of drug induced liver injury

V A J Maria, R M M Victorino Gut 1997;41:534-540

## Conclusions

- **Positive lymphocyte transformation test in as many as 56% of patients suggests immunological mechanisms far more frequent in drug hepatotoxicity than previously assumed**
- **T-cell reactivity represents additional evidence in favour of its involvement in liver injury and is important for defining mechanisms**



# Lymphocyte transformation test (LTT)

Detection of ximelagatran and/or melagatran-specific lymphocytes in peripheral blood of treated patients

## *Group 1 - patients*

13 cases with treatment discontinued at ALT > 4 x ULN

## *Group 2 - patients*

8 cases with treatment continued after ALT > 4 x ULN

## *Group 3 - controls*

15 subjects without ALAT elevations (matched with groups 1 and 2)

## *Group 4 - controls*

10 unexposed subjects

**One subject from group 1\* and one from group 2 were positive to ximelagatran. The group 1 subject was also positive to melagatran. All control subjects were negative (=100% specificity of the assay).**

\*HLA-DRB1\*0701 carrier

*Kindmark et al 2007*

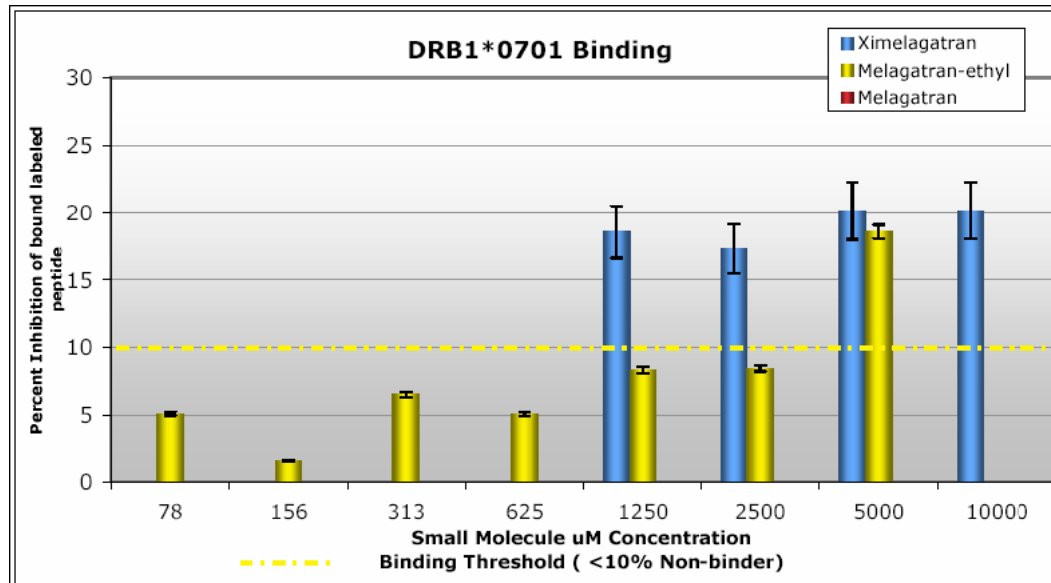


# Drug affinity studies, EpiVax (Providence, US), were performed to validate results from the pharmacogenetic study (EXGEN)

**Competitive binding to soluble MHC molecules** encoded by HLA-DRB1: \*0101, \*0401, \*0701 and \*1501 in the presence of peptides bound to these MHC molecules.

**Ximelagatran** and its metabolites **melagatran** and **ethyl-melagatran** were tested.

# Strongest association observed with ximelagatran and HLA-DR7



Strong binders: >50% inhibition of peptide binding

Weak binders: 10-50% -"-

Non-binders: <10% -"-

*Kindmark et al 2007*

**Ximelagatran may form antigenic structure via direct binding to HLA-DR7?**



# SUMMARY: Indicators of an immune mechanism

- Time to onset of ALAT elevation: 1-6 mo
- Incidence of cases: 7.9%
- Species specific: No ALAT elevations in animal models
- Recovery upon continuous treatment: induction of tolerance?
- Evidence of drug specific T-cells:
  - Melagatran classified as a moderate skin sensitizer in GPMT
  - LTT+ in 2/7 occupationally exposed workers with skin reactions
  - LTT+ in 2/21 cases with ALAT<sup>↑</sup>. 100% specificity.
- MHC-associations: HLA-DRB1\*0701 identified by pharmacogenetics and confirmed by affinity studies performed by EpiVax.
- Monocyte activation – CSF1R shedding as a treatment effect shown by proteomics data and verified by *in vitro* studies



# Planned/ongoing activities

- HLA-DRB1\*0701/DQA1\*02/CD4+ Tg-mouse model being developed
- Diet restriction models – rat and Tg-mouse for further investigation of the pyruvate hypothesis



# Ximelagatran Biomarker studies

## Acknowledgements

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### **Proteomics/Metabolomics Study**

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Mira Hannula, Hugh Salter, Kerstin Nilsson

And many other colleagues



# Thank You !