Drug-Induced Liver Injury

What Kind of a Biomarker Do We Need?

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DR. AVIGAN: Thank you, John (Pears), thank you Lana (Pauls), and thank you, John (Senior). I have a very significant challenge today. I'm going to try to be as expeditious and brief as possible. My challenge is to talk about the discovery and the development of biomarkers for drug induced liver injury as well as susceptibility to DILI and intertwine this with what our process of risk assessment and risk management during the lifecycle of a drug.

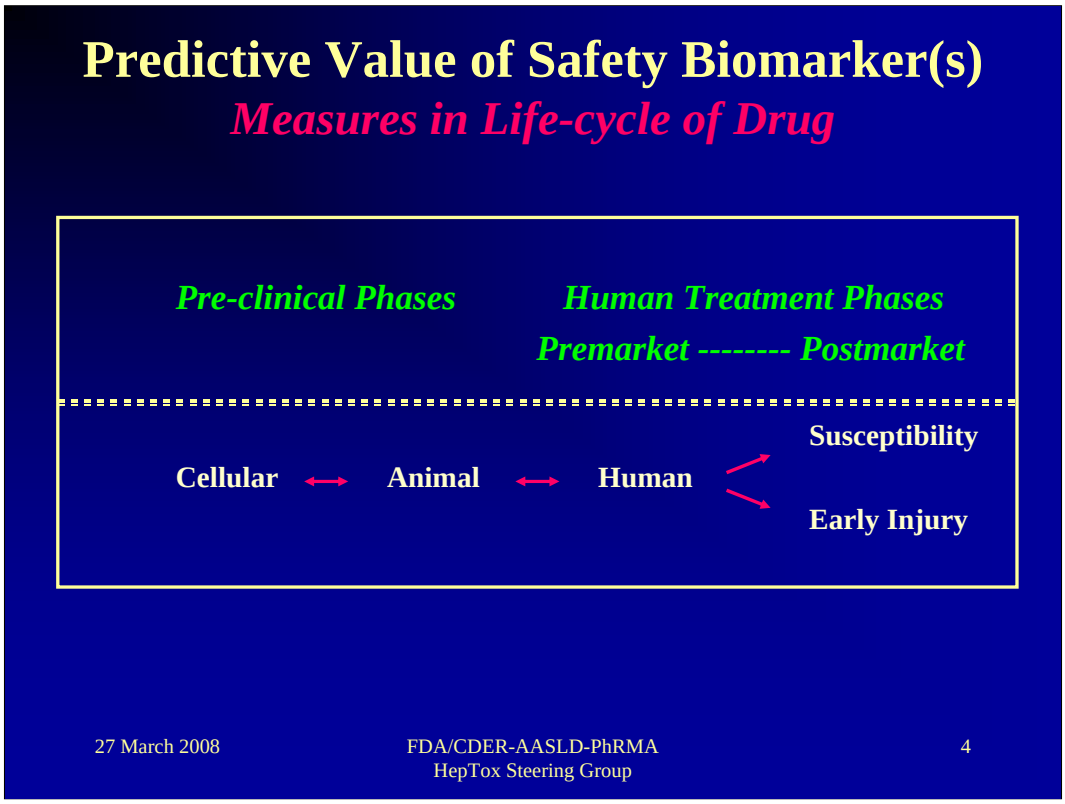
Overview of Presentation

- Predictive Value of Safety Biomarkers during Drug Life-cycle
- Biomarkers of Drug Toxicity & AE susceptibility
  - performance requirements & examples
- Elevated ALT & DILI risk
- Biomarkers of DILI Susceptibility
  - studies & challenges
- Summary & Conclusion

This is an overview of my presentation. These slides will be posted on the web and you'll have an opportunity to look at them more carefully.

As you all know, risk assessment of drugs is a lifecycle challenge. During different phases of the cycle, when a safety issue is identified different questions about drug-related risk come to the fore. Early on, before approval of a product, if a drug safety problem is identified, a question that needs to be addressed is whether the drug development program should proceed or should be discontinued. When a toxicological signal emerges in the pre-clinical phase, there's a question about what the potential mechanisms of toxicity are, and whether these could be avoided in a human population. During clinical trials, there is a need to protect study subjects from serious outcomes by appropriate patient enrollment and monitoring practices.

After approval, if there is a safety problem, there's a question of how to effectively manage drug-related risk, either by appropriate patient selection for treatment or by adequate monitoring to detect early organ injury at a time when changing drug dosaging or discontinuing treatment would mitigate risk.



It follows that the availability of reliable safety biomarkers or measures that could answer these questions concerning drug related risk at each of the stages that I mentioned, would be a very useful set of tools for drug development and for risk management. In addition, they might play an invaluable role in the selection and management of clinical trial subjects. It should be emphasized that there would be two general categories of these biomarkers - those that would identify the subset of patients who are highly susceptible to a drug-related adverse event prior to drug exposure and those that would indicate early toxicity destined to become more severe or life-threatening at a time when drug dosage adjustment and/or cessation of treatment would effectively mitigate risk.

Predictive Value of Safety Biomarker(s)

Valid biomarker: A biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the test results. The classification of biomarkers is context specific. Likewise, validation of a biomarker is context-specific and the criteria for validation will vary with the intended use of the biomarker. The clinical utility (e.g., predict toxicity, effectiveness or dosing) and use of epidemiology/population data (e.g., strength of genotype-phenotype associations) are examples of approaches that can be used to determine the specific context and the necessary criteria for validation.

Guidance for Industry:
Pharmacogenomic Data Submissions; March 2005

The concept of diversity of drug safety biomarker roles during the life-cycle of a drug is captured in the Guidance for Industry document that was put out by the FDA in 2005. This guidance highlights the idea that the classification of biomarkers is context specific. As a result, the criteria and methods that would be employed to validate biomarkers would also be context specific and would vary with the intended use of the biomarker. We'll come back to this point later.

Predictive Value of Safety Biomarker(s)
Concepts

- **Biomarkers might be used to**
  - **establish differences of risk potential between drugs**
  - **predict serious vs mild vs no toxicity**
  - **identify 'susceptible' vs 'tolerator' vs 'adaptor' pts**
- **Utility of biomarker to predict one characteristic of toxicity or AE risk in one phase of a drug's life-cycle may not be relevant for another**
- **Discovery & validation of biomarker(s) to manage risk depends on clinical correlation and comprehensive risk assessment**

Drug safety biomarkers might be used in a number of ways. I've listed what these are, and I'm not going to read every line of the slide. The important point is that the utility of biomarkers to predict one characteristic of toxicity or AE risk in one phase of a drug's lifecycle may not be relevant for another. In addition, clinical correlation is a critical underpinning both for biomarker discovery and validation.

Signal Detection & Risk Assessment
Challenges

- **Rare serious/life-threatening adverse events may not be seen in clinical trials**
- **Presently, there is a paucity of validated biomarkers that reliably predict *which drugs* will cause idiosyncratic drug AEs, portend serious injury *before it has occurred*, or indicate who are *susceptible patients***
- **How a weak signal of mild injury in a small test population will 'play out' after marketing may be difficult to predict**

With regards to drug safety signal detection and risk assessment one of the great challenges that we face is that rare, serious or life threatening adverse events may not be seen in clinical trials. Also, there's really a paucity of validated biomarkers that reliably predict which drugs will cause idiosyncratic drug adverse events, including DILI, portend serious injury before it has occurred or indicate who are the susceptible patients.

So we really are playing a kind of game of uncertainty. Typically, before its marketing we don't know how a drug safety signal may play out in a larger exposure population and with the limited safety information that may be available at the time when a NDA is being considered for approval it may be very difficult or impossible to predict the right answer.

Mild AE Signal --- Rare serious events?

Examples

• Myopathy/↑CPK:	Rhabdomyolysis
• QT lengthening:	Torsade de Pointes
• ↑ALT:	Idiosyncratic ALF
• Increased creatinine:	Renal failure
• Urticaria:	Anaphylaxis
• Rash:	Serious skin reaction
• Leucopenia:	Agranulocytosis

A typical scenario which sometimes emerges from the evaluation of clinical trial safety data, is one in which there are imbalances of mild drug-related idiosyncratic adverse events between the randomized groups with an excess of these events in the patients who have received the test drug compared to those who have received placebo or other treatment arms. I've listed some examples of the mild or non-life threatening injuries of interest in the left column. In gauging the clinical impact of this finding, the critical question that must be answered is what would be the level of risk for the most severe or life-threatening forms of these drug-related injuries, which I've listed in the right column, in a larger exposure population after marketing of the drug has begun.

At the population level we are concerned about an iceberg effect in which the size of its tip represents the most severe forms of drug toxicity. Indeed, the iceberg tip may be broad, representing significant risk for serious events, or conversely it might be very narrow or negligible, representing a very low level of drug-related risk. At the initiation of marketing of the drug we may not be able to predict how this tip will turn out. In such an instance an effective pharmacovigilance strategy would have to be instituted in order to answer the question.

Idiosyncratic forms of DILI

Assessment of Population Risk

Interplay between susceptibility of individual and external environmental/disease factors

- **3 possible drug safety responses: 'tolerators', 'adaptors', susceptibles'**
- **'Tolerators' and 'adaptors' often seen in clinical trials; whether 'susceptibles' will occur after marketing may require study/surveillance of larger treatment population**
- **Difficult to predict without biomarkers of susceptibility**
- **Biomarkers of early injury may not distinguish 'adaptors' from 'susceptibles'**

To characterize inter-individual differences of susceptibility to idiosyncratic forms of DILI, we need to take into account the interplay between individual characteristics and external and environmental or disease factors. There are three possible categories of responders to a drug known to cause idiosyncratic DILI. Most people are 'tolerators' – these individual tolerate exposure to the drug without developing any form of liver injury. A smaller group of patients are 'adaptors' - these individuals develop drug-induced mild forms of liver injury which typically will resolve even when treatment with the inciting drug is continued. The smallest category of responders is comprised of 'susceptibles' who progress to serious, life-threatening liver toxicity.

In the clinical trial phases of drug development prior to marketing with typically only a few thousand patients exposed to the agent we often only see the 'tolerators' and 'adaptors'. Without adequately predictive biomarkers it would be difficult or simply impossible to predict who will be the 'susceptibles'.

Predicting Dosing & DILI Susceptibility Factors
Common Biological Complexities

- **Defect(s) in a metabolic pathway(s) associated with injury from a drug may not be generalizable to other drugs**
- **Redundancies in metabolism, transport, adaptation and regeneration may be overwhelmed only by particular combinations of defects**
- **Often more than one genetic variant in a population may lead to changes in optimal drug dosing, susceptibility to idiosyncratic AEs or loss of adaptation**

In the search for such biomarkers there are important biological phenomena that we have to consider. First, a defect in a single or set of metabolic or cellular pathways that is the basis of a drug-related injury may not be generalizable to other drugs. Second, hepatocellular detoxification, repair and regenerative systems often are redundant, providing most of us with extra layers of protection from toxic xenobiotics, or their metabolites. Unfortunately, such redundancies may hinder the discovery and elucidation of the specific pathway changes that underlie serious idiosyncratic DILI in rare individuals.

Sometimes different genetic variations in different genes may cause the same liver injury phenotype because of the potential for shared roles in the same set of cellular functions. In other cases, conversely, an identical genetic variant in different individuals may yield different forms or degrees of liver injury because of the convergence of other modifying factors in which there may be important differences. No doubt, these unexplored biological complexities will throw curve balls at us during the very challenging road we must go down to untangle the DILI problem!

Risk Management Strategies

Scenario 1

- **Risk for serious AE identified *prior to/soon after* marketing; Serious outcome may be prevented by pt monitoring or observation; Early toxicity informs drug d/c or dose adjustment; For some drugs RiskMAP critical for favorable benefit/risk.**
- ***Examples*: Clozapine-induced agranulocytosis; DILI linked to dantrolene, felbamate, zileuton, zafirlukast, tolcapone etc.**
- ***RiskMAPs*: Clozapine - 'No blood, No drug'; Hepatotoxic drugs - ALT monitoring, d/c drug if ALT elevated (effectiveness not demonstrated although often instructed in label).**
- ***Could evaluate:* Incidence of WBC suppression, rates of agranulocytosis & infections, WBC monitoring effectiveness, Rates of clinically serious DILI or drug-induced ALF**

When we fast forward to how biomarkers could be utilized as tools for risk management of drug treatment in clinical practice, there really are two scenarios that we need to consider.

The first scenario is one in which a drug-induced serious adverse event can be prevented by regular patient monitoring using a biomarker of early drug related toxicity. In this case, risk can be effectively mitigated with a biomarker by detection of early toxicity at a time when one can either remove the drug or adjust its dose in a timely manner before serious clinical consequences have emerged. An example is the regular white cell count monitoring that is required in the risk management of all patients using clozapine - the so called 'no blood, no drug' program- to prevent life-threatening agranulocytosis. Unfortunately, because of limitations in specificity and predictive power, in certain instances it has been difficult to demonstrate the utility of regular ALT monitoring for the purpose of preventing clinically serious DILI.

Risk Management Strategies

Scenario 2

- **Risk for serious AE in definable sub-population; Serious outcome may not be prevented by Rx discontinuation; Benefit/risk favored by preventing use or special dosing in vulnerable patients.**
- ***Examples*: isotretinoin & thalidomide in pregnancy; Carbamazepine: SJS/TEN & HLA B*1502; Abacavir: hypersensitivity & HLA B5701.**
- ***RiskMaps*: iPLEDGE and STEPS registries require negative pregnancy tests prior to initial Rx & refill and provide instructions for contraception.**
- ***Could evaluate*: precise numbers and rates of pregnancy exposures, pregnancy test result linkage to Rx, root causes of pregnancy exposure and outcomes; episodes of hypersensitivity with HLA screen.**

The second scenario where biomarkers might be used in risk management of drug treatment in clinical practice is one in which increased risk for an idiosyncratic serious adverse event is not reliably reversed by discontinuation of treatment, even when toxicity is detected early after the initiation of drug treatment. In this case, risk can only be mitigated with an appropriate biomarker by avoidance of use or appropriate dose adjustment, prior to initiation of treatment, by identifying the subset of susceptible individuals. A classic example of this scenario is the requirement for regular pregnancy testing in females of child-bearing potential in the risk management of isotretinoin and thalidomide. Similarly, HLA testing has been advocated prior to initiation of treatment to preclude individuals with specific HLA allelic variants who are susceptible to the development of carbamazapine-induced serious skin reactions or abacavir-associated systemic hypersensitivity from receiving these agents. In a moment you will hear about some research that has been conducted to identify pharmacogenomic susceptibility biomarkers for idiosyncratic DILI associated with particular drugs.

Biomarkers of Drug-Related Toxicity/AE Susceptibility
Requirements for risk management

- **Have sufficient negative and positive predictive power to inform treatment decisions**
- **Are validated for performance in target patient population**
  - **generalizable**
- **Have advantage over standard practice & diagnostic testing**
  - **superior predictive power**
  - **patient/physician preference, economical, amenable to scaling up & high compliance, infrastructure support**
  - **screen of very rare events justified if severe clinical outcomes are prevented**
- **Have solid basis; future biological discoveries unlikely to invalidate, although may be refined**

From these two scenarios it follows that to be effective tools for risk management, drug risk biomarkers must have sufficient negative and positive predictive value to inform treatment decisions. In addition, they need to be validated for their performance characteristics in the intended treatment population and these results should be generalizable after marketing of the product. No matter what our role is as regulators, in the real world, patient care decisions in clinical practice that are guided by biomarker testing results should provide a clear advantage over standard practice. Widespread implementation of biomarker testing will only take place if both patients and the physicians perceive this advantage. In addition, there should be a sound economical basis for their use as well as availability of the necessary infrastructural support to enable consistent testing across the appropriate treatment populations. Even for the purpose of preventing rare drug-related adverse events, such screens or monitoring programs might be justified if these events are serious and life-threatening.

Biomarkers of Drug-Related Toxicity/AE Susceptibility

Definitions of test characteristics

Occurrence of Adverse Event

Test

	+	-	Predictive Power (PP)
+	TP	FP	Pos PP: TP/TP+FP
-	FN	TN	Neg PP: TN/TN+FN
Total (Tot): TP+FP+TN+FN	Sensitivity: TP/TP+FN	Specificity: TN/TN+FP	Accuracy: TP+TN/Tot

Here is a matrix which defines test characteristics of drug safety biomarkers. When we think about the utility of a new biomarker in an intended drug treatment population, it's useful to consider the four elements of this matrix. Using clinical correlation, these elements are the numbers of true positives, false positives, false negatives and true negatives. From their relative proportions one can calculate the biomarker's sensitivity and specificity characteristics. With regards to safety, what we really want are biomarkers that minimize false negative tests but have maximal sensitivity for the detection or prediction of the adverse events of interest.

With regards to toxicity, we also want biomarkers for which the proportion of false positives is low in order to enhance the positive predictive power of the testing. Simply put, the test will be less valuable if it does not discriminate serious drug related toxicity from trivial self-limited toxicity or injuries that are caused by non-drug etiologies.

Biomarkers of Drug-Related Toxicity/AE Susceptibility

Test characteristics & utility

1. ***Good* Sensitivity/Positive & Negative Predictive Power**
   - **High test specificity**
   - **Higher prevalence of test positive affected individuals (‘*true positives*’) compared to non-affected individuals (‘*false positives*’) in population**
   - **Useful both for tests of susceptibility or clinically significant toxicity**
2. ***Good* Sensitivity/Negative Predictive Power**
   ***Poor* Positive Predictive Power**
   - **Low test specificity**
   - **Prevalence of ‘*true positives*’ typically low in comparison to prevalence of ‘false positives’ in population**
   - **May be useful as markers of AE susceptibility but limited in utility if not complemented by other independent markers; generally not useful as indicators of clinically significant toxicity**

Based on these parameters, a biomarker in development may end up in one of four possible categories of utility. The first category characterizes ideal biomarkers for both drug-related toxicity as well as individual susceptibility to idiosyncratic adverse events. Biomarkers in this category, as I already described, have high sensitivity and in addition both high positive and negative predictive power.

A second biomarker performance category is marked by good sensitivity but poor positive predictive power. In monitoring for DILI Elevations of serum ALT levels pretty much fit into this category since the isolated test result does not discriminate liver injuries that are destined to progress to life-threatening levels from those which are minor and self limited.

Biomarkers of Drug-Related Toxicity/AE Susceptibility
Test characteristics & utility

3. ***Good* Positive Predictive Power & Specificity**
   ***Poor* Sensitivity & Negative Predictive Power**
   - **May be useful as predictors of serious toxicity in patients & guide for drug development; Not reliable as screens for AE susceptibility unless complemented by other tests to reduce overall *false negative* rate.**

4. ***Poor:* Positive & Negative Predictive Power**
   - **Not useful**

Because of the short time, I'm not going to touch on these other categories. You may want to look at the slides later.

AE Susceptibility Biomarkers

Pathway for development & utilization

- **Strategy to inform appropriate dosing or patient selection for treatment to mitigate risk**
- **Discovery/exploratory biomarker studies**
- **Study validation/replication**
- **Review/critique**
- **Define appropriate role(s) for risk management**
- **Develop routine testing process and necessary support structure**
- **Communicate with stakeholders including health care providers**
- **Evaluate effectiveness of biomarker tests in 'real world' setting**

Although the search for pharmacogenomic biomarkers that indicate heightened susceptibility to idiosyncratic drug-related adverse events has only recently been undertaken, there are already a number of examples which have been validated and incorporated into FDA product labeling. In the pathway for development and utilization of these types of biomarkers the product labeling step is just one milestone among a series of steps that I have listed in this slide. The process begins with creation of a strategy to inform appropriate dosing or patient selection and continues with performance of both exploratory and replication studies that measure the sensitivity, specificity and predictive values of new markers. The next step is review and critique of all pertinent data by academics and regulators to establish the groundwork for their implementation as risk management tools. An essential step is to communicate with all appropriate stakeholders including health care providers in order to gain feedback and enhance acceptance of newly developed biomarkers by the clinical community. Finally, it is critical that studies be undertaken to evaluate the effectiveness of these biomarkers in a real world setting.

AE Susceptibility/Pharmacogenomic Biomarkers

Examples in FDA Approved Labels

Drug(s)	Biomarker	Test	Adverse events	Product label
Thioridazine	**CYP 2D6** ***PMs***	~12 variants; 2 rearrangements	Vent. arrhyth.	Use contraindicated
Voriconazole	**CYP 2C19** ***PMs***	Alleles *2A, *2B, *3	Toxicity	Info
Imuran & 6-MP	**TPMT** ***Low activity***	Alleles *2, *3, *3C	Serious leukopenia	Homozyg: other treat; Heterozyg: use with caution
Irinotecan	**UGT1A1** ***Low activity***	Allele *28	Diarrhea & neutropenia	Homozyg: Low initial dose
Warfarin	**VKORC1/ CYP 2C9**	-1639 G>A / Alleles *2, *3	Overdose & bleeding	consider low initial dose

These are some examples of the adverse event susceptibility pharmacogenomic biomarkers that have already been incorporated into FDA approved labels. In the table of examples shown in this slide the labeled instructions for actions to be taken based on biomarker test results appear in the far right column with the names of the specific corresponding drugs in the far left column. Most of these biomarkers reflect pharmacogenomic variants of genes which encode enzymes that regulate the inactivation or clearance of pharmacologically active parent drugs or their metabolites. In each case, treatment instructions are influenced by the size of the therapeutic index and the implied severity of a safety outcome if enzyme levels are altered. For example, TPMT is an enzyme that regulates the metabolism of Imuran and 6-MP. This enzyme is in an alternate pathway for disposal of these drugs; the other pathway leads to the formation of 6-thioguanine; homozygotes with low TPMT activity variant alleles, are prone to developing very high 6-thioguanine levels and consequently, serious leukopenia. Because this risk is very high, the labels contraindicate usage of these products in the low TPMT activity homozygotes, suggesting that their use should be altogether avoided in these patients.

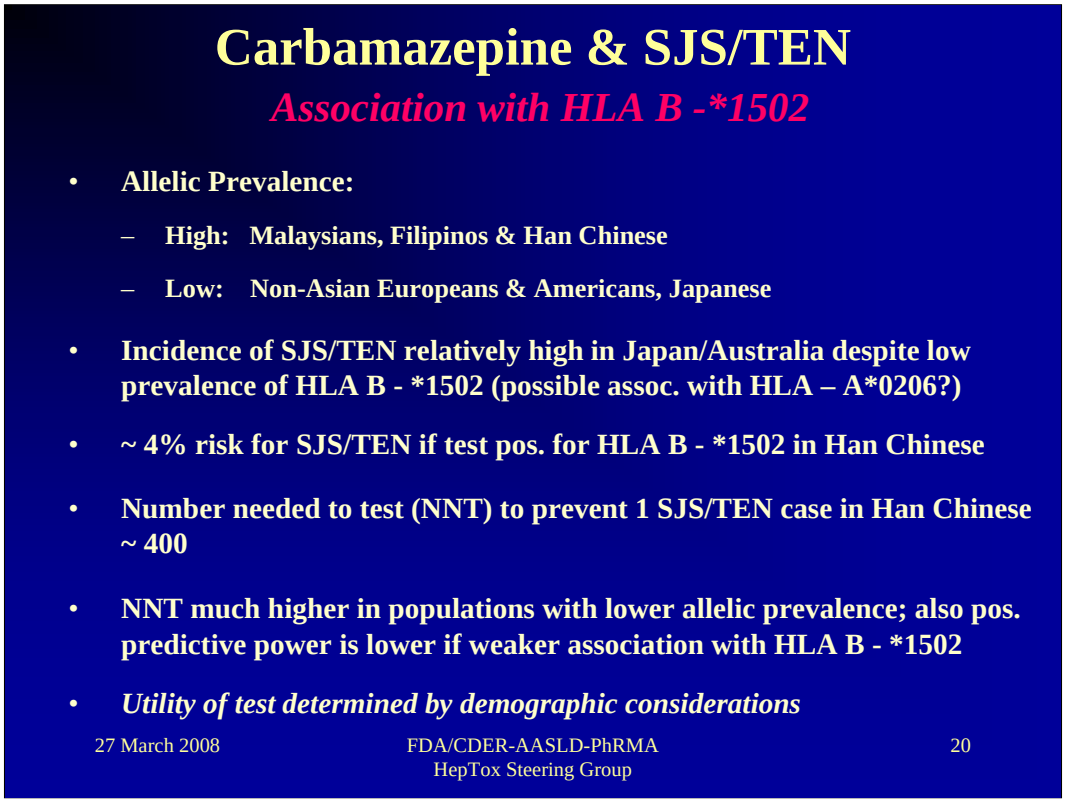
Warfarin labeling lists three genomic variants, one in the VKORC1 gene and the other two in CYP2C9, which influence optimal warfarin dosing requirements. This list of genomic variants is a very small subset of all the genomic variants both in these two genes as well as others which influence warfarin activity and appear at different prevalence rates in the human population. Why these particular variants have made the currently approved label, whereas others have not so far, is based on a number of factors including their prevalence in specific demographic populations, and their relative influence on inter-individual dosing variability. We'll come back to warfarin in a moment.

Drug AE Susceptibility/HLA Biomarkers

Examples: Serious hypersensitivity or skin reactions

Drug	HLA	Adverse event	Allelic Prevalence	Product Label
Abacavir	HLA-B*5701	Moder./severe hypersensit. reactions	Demographic variability Caucas. ~ 5%	*HLA association not described*
Carbamazepine	HLA-B*1502	SJS/TEN	Demographic variability Han Chin. ~ 8%	Boxed Warn. *test pos. pts. not treated unless benefits > risk*

Another group of drug-related AE susceptibility biomarkers which I want to touch on are HLA allelic biomarkers which indicate risk for hypersensitivity reactions. I have listed two examples. In the case of carbemazepine there are demographic populations in which the biomarker is especially useful. In both the cases of abacavir and carbemazepine the respective HLA allelic biomarkers for susceptibility to these drug reactions generally demonstrate low false negative rates in certain demographic groups. However, as you will seen in a moment, most treated patients with these biomarkers don't get the adverse reactions.



Let me now drill down to carbamazepine and product labeling instructions concerning the HLA-B*1502 biomarker. The FDA approved label states that testing for this marker prior to initiation of treatment is useful in certain demographic groups such as Asian sub-populations. In these groups, if there is a positive test for the HLA allelic variant, the drug should not be used because of the substantial risk for Steven Johnson Syndrome and Toxic epidermal necrolysis.

Part of the rationale for the instruction to selectively test only certain groups is that the prevalence of HLAB-*1502 varies widely in different demographic populations. For example, it is high in Chinese but low in non-Asian Europeans. The important point is that in Han Chinese - a high risk group – treatment with carbemazepine is linked to a four percent risk for a serious skin reaction in patients who test positive for the allele. This predictive power can be calculated from the rate of serious skin reactions in carbemazepine treated patients and the number of treated patients in the population. With back of the envelope math, you need to test about 400 Han Chinese patients to prevent 1 case of SJS, because the prevalence of that allele in that population is about 5 percent.

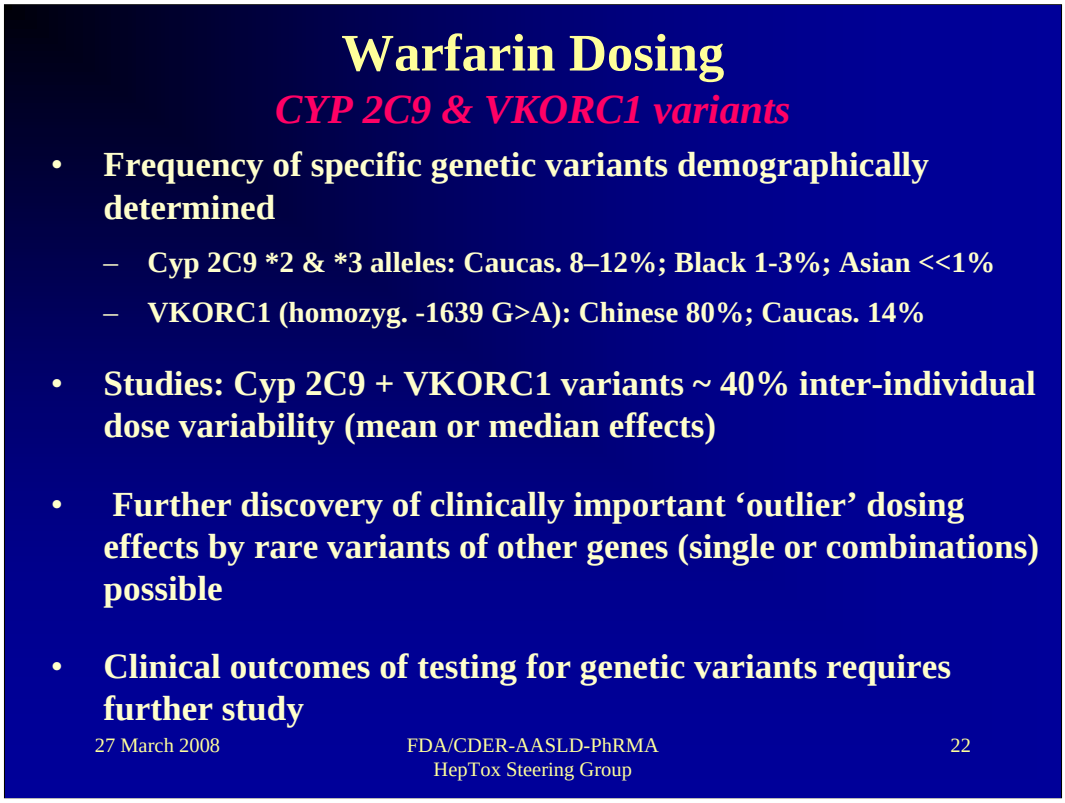
Testing patients before treatment seems to be a reasonable risk management procedure in a population with such a high prevalence for the biomarker because of the severity of these skin reactions. The take home message is that utility of such testing is strongly influenced by demographic considerations.

Warfarin Dosing

Pharmacogenomic biomarkers

- **Warfarin associated bleeding has public health impact; NHAMCS: over 50,000 patients hospitalized per year in US (1999-2003)**
- **Variability in optimal dosing affected by both non-genetic factors (age, weight, liver disease, drug-drug interactions, etc.) & genotype**
- **Genetic variants influence warfarin dose variability**
  - **Reduced CYP 2C9 activity (e.g. Alleles *1, *2, *3)**
  - **Reduced VKORC1 activity (e.g. nucleotides -1639, 1173) or changed mRNA levels (e.g. Haplotypes 'A' vs 'B');**
  - **Variant Clotting factors II, IV IX X; Protein C, S, or Z deficiencies**
  - **Variants of γ– Glutamyl carboxylase (e.g. microsatellites in intron 6)**
  - **Apolipoprotein E isoforms E2, E3 & E4: Different Vitamin K uptake in liver**

Because of the frequency of over- or under-dosing of warfarin, adverse events linked to this agent continue to pose a large public health problem. As I already mentioned, warfarin effects on vitamin K activity and coagulation factors are influenced by complex sets of non-genetic and genetic factors. I have listed some of the genetically determined biochemical or enzymatic functions which modify or are affected by warfarin's action on INR. These regulate Vitamin K activity, the metabolic clearance of warfarin itself, affect warfarin's substrate – VKORC1 which replenishes vitamin K activity through a reduction step, or modulate the efficiency of the γ-carboxylation reactions necessary for clotting factor activity. In the human population, for each of these steps, there are a number of allelic variants at different genetic loci marked by altered activity which may modify optimal warfarin dosing. Therefore, in addition to the specific cytochrome 2C9 and VKORC1 promoter allelic variants that have been listed in the product label as influencing warfarin dosing, there are other pharmacogenomic variants that may also play a significant role in the optimal dosing of some treated patients.



Because of variable prevalence characteristics of the labeled VKORC1 and CYP 2C9 allelic variants in different demographic groups these particular biomarkers have relevance in informing warfarin dosing decisions in certain populations. For example, the prevalence of the low activity Cyp 2C9 *2 and *3 alleles is high in Caucasians but low in Asains. Conversely, the prevalence of the homozygous form of the low activity VKORC1 -1639 G>A variant is very high in Chinese and lower in Caucasians. Together, these 2C9 and VKORC1 variants account for up to 40 percent of the inter-individual dose variability in demographic groupings in these particular populations.

Further discovery of clinically important outlier dosing effects by rare variants of other genes is possible and inevitably will occur. We can expect new pharmacogenomic studies, further discovery and further adjustments of biomarker testing instructions that will inform optimal warfarin dosing in the future.

DILI Biomarker Measurements

Questions for Development & Implementation

- **Are markers of early hepatotoxicity accurate predictors of serious injury before it has occurred?**
  - **– may enable timely discontinuation of drug**
  - **– prognostic markers in individual patients**
  - **– risk in treatment population**
- **Are markers of an individual's susceptibility to DILI reliable?**
  - **– utility in risk management for treatment decision or dosing**

Let me shift back to a discussion of DILI. There are important questions that need to be addressed surrounding the utility of DILI predictive biomarkers as tools for risk management – markers of liver injury or individual susceptibility to idiosyncratic reactions. I've listed these and from my previous comments they should come as no surprise.

Signals of Elevated Serum ALT
How have they 'played out'?

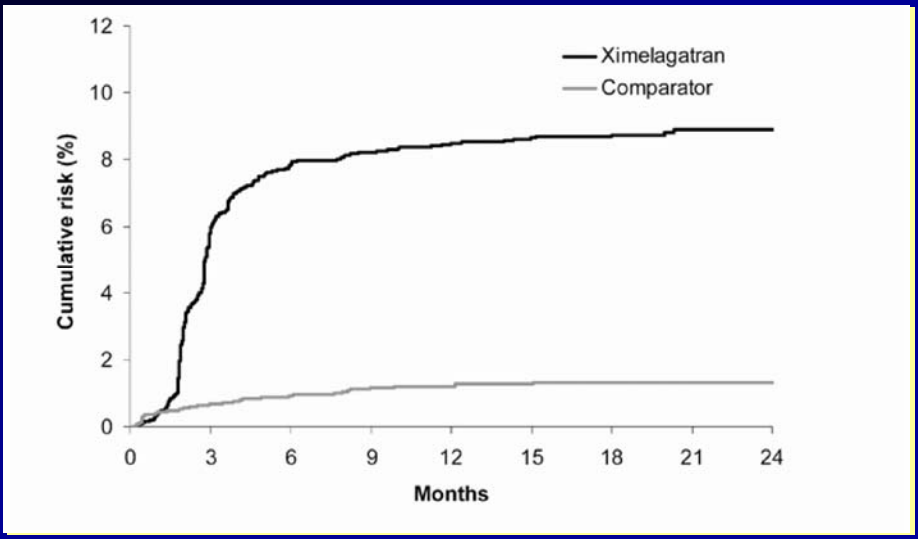
2 Contrasting Examples

- **Ximelagatran**
- **Lovastatin**

We know that during clinical trials drug associated isolated elevations of serum ALT levels may or may not signal risk for more serious liver injuries in other treated patients. The uncertainty of risk for severe reactions poses a conundrum during drug development. Serum ALT signals have played out differently for different drugs! To make this point I highlight two contrasting examples – ximelagatran which has been linked to a full range of DILI severity and lovastatin which has demonstrated a negligible association with serious causally related liver injury outcomes.

ALT > 3X in Long Term Trials

Cumulative Risk Over Time



www.fda.gov/ohrms/dockets/ac/04/briefing/

Because of time I will pass over the next few slides that demonstrate these contrasting effects but the slides will be available for your perusal on our website.

Severe Liver Injury - Long term Rx

All randomized patients

- **Concurrent increase of total bilirubin > 2x ULN within 30 days of ALT rise > 3x ULN**
- **37/6,948* (0.5%) in ximelagatran Rx groups vs 5/6,230 (0.08%) in warfarin Rx groups**
- **Relative risk 6.6 (95% CI 2.6 – 16.9)**
- **3 liver injury associated deaths**

* Mean Exposure in Long-term Experience (LTE) trials – 357 days

www.fda.gov/ohrms/dockets/ac/04/briefing/

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(shown quickly without comment)

Adaptation in Ximelagatran-treated patients who developed ALT > 3x ULN

	Study drug discontinuation=No xULN				Study drug discontinuation=Yes xULN				
Max(ALAT)	≤1	(1,2]	(2,3]	>3	≤1	(1,2]	(2,3]	>3	Total
>3xULN	84	10	4	1	101	4	3	4	211
>5xULN	97	5	3	1	76	6	0	4	192
>10xULN	36	3	0	1	73	7	0	8	128
Total	217	18	7	3	250	17	3	16	531

Follow-up ALAT measurements include those made at local laboratories

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(shown quickly without comment)

Lovastatin

Potential link to DILI

- **Clinical trial databases (pre & post-marketing)**
  - **– *Original NDA studies*: 1.5% pts - ALT > 3X ULN & interrupted drug; all asymptomatic; 5/9 pos re-challenge**
  - **– *EXCEL*: 8,245 pts; Pbo controlled 48 wk study; Consecutive ALT > 3X ULN: Pbo – 0.1%; Lovastatin 80 mg/d – 1.5%; 40 mg/d – 0.9%; 20 mg/d – 0.1%; No severe DILI**
  - **– *AFCAPS/TexCAPS*: 6,605 pts; Pbo controlled study; mean f/u 5.2 yrs; Consecutive ALT > 3X ULN: Pbo – 0.34%; Lovastatin 20 mg/d or 40 mg/d – 0.52%; No severe DILI**
- **US ALF reporting rate in AERS (first 4 yrs of marketing): ~ $2/10^6$ person-yrs of exposure – close to background rate**

Because of time I will pass over the next few slides that demonstrate these contrasting effects but the slides will be available for your perusal on our website. So again you can look at the slides.

(shown quickly without comment)

Lovastatin & Potential for DILI

Patients with Pre-existing Liver Disease

- ***N. Calif. Kaiser Permanente Study*:***
  - **– Retrospective cohort study design; Lovastatin exposed vs non-exposed time**
  - **– 93,106 adult enrollees; baseline elevated ALT or liver disease dx**
  - **– 13, 491 exposed to lovastatin, median 9 mo.**
  - **– Outcomes: 1^0 - elevated ALT + Bili (Hy's rule); 2^0 - Liver injury & Cirrhosis/LFailure**
  - **– Analysis: Lovastatin treated pts significantly less likely to develop 1^0 or 2^0 outcomes (Incidence Rate Ratio, 1^0 Outcome, univariate analysis ~ 0.28, multivariate analysis ~ 0.26)**
  - **– Limitations of study: Potential channeling bias due to physician treatment preferences; Disparate liver conditions not separately analyzed; Potential for misclassification of liver conditions including NAFL/NASH**

*FDA Review by S. Bezabeh, www.fda.gov/ohrms/dockets/ac/07

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(shown quickly without comment)

Lovastatin & Potential for DILI

Pre-existing Elevated Serum Transaminases

University of Indiana Prescription - Lab Data Linkage Study*

	Cohort 1[tt]	*Cohort 2[tt]*	*Cohort 3[tt]*	*p values*
	Baseline ALT + lovostatin (n = 135)	**Baseline ALT nl + lovostatin (n = 620)**	**Baseline ALT – lovostatin (n = 2245)**	**Cohort 1 vs Cohort 3**
Elevated ALT < 10X ULN	**6.6%**	**3.0%**	**11%**	**p = 0.2**
Elevated ALT > 10X ULN	**0%**	**0.3%**	**5.5%**	**p < 0.01**
ALT > 3X ULN Bili > 2X ULN	**0%**	**0%**	**3.0%**	**p = 0.3**

[tt]12 Month period of follow-up; Exclusions: Pts with EtOH abuse or presence of viral hepatitis B or C serologic markers

*Vuppalanchi et al., Am. J. Med Sci., 2005; FDA Review by E. Craig, www.fda.gov/ohrms/dockets/ac/07

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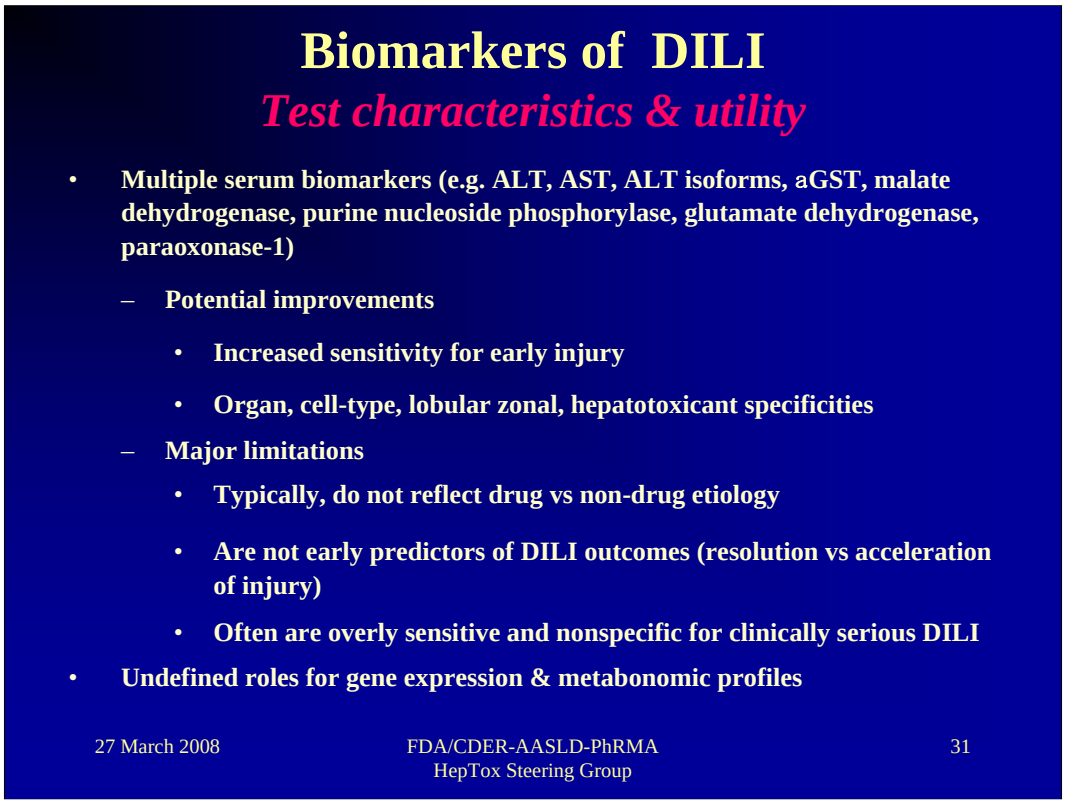
(shown quickly without comment)



As new biomarkers of DILI will be developed, we have to take stock of the problem of limited specificity that characterizes a number of serum biomarkers of hepatocellular injury which are listed on this slide and have been studied to date. Most of these reflect spillage of hepatocellular enzymes from the cellular cytoplasm or mitochondria. In some instances, when used alone or in certain combinations as panels, these biomarkers may demonstrate marginally superior sensitivity compared to total serum ALT for the detection or early liver injury or point to a specific injured cell type or type of hepatotoxicant exposure. Nonetheless, these DILI biomarkers have significant limitations. They do not appear to discriminate between drug and non-drug etiologies of liver injury, and more importantly they do not distinguish DILI events destined to progress in 'susceptibles' from self-limited toxicity events in 'adaptors'.

Once again, as a biomarker the sensitivity of elevated serum ALT for DILI is reasonably adequate. It is its limited specificity which has been problematic. To date, despite some improvements, the new DILI biomarkers do not appear to have specificity for toxic events destined to evolve into clinically serious DILI.

Biomarkers of Acute Kidney Injury (AKI)

Test characteristics & utility

- **Serum creatinine not sufficiently reliable & sensitive**
  - **May not change until 50% reduction in kidney function**
  - **After AKI, increase may take few days**
  - **Varies by age, gender, body mass, muscle metabolism & hydration status**
  - **Does not discriminate etiology/type of renal injury**
- **Promising AKI biomarkers - examples**
  - **NGAL (plasma/urine); KIM-1 (urine); Cystatin (plasma); β-2 microglobulin (urine)**
  - **Increased sensitivity for early injury detection is advantageous**
  - **Combinations may indicate pathologic sites & time intervals after AKI**
  - **May be useful as pre-clinical pharm-tox measures & for early human trials**
  - **Possible expanding role in clinical trials & dosing of drugs**

In contrast to elevated serum ALT as a biomarker for DILI, elevation of serum creatinine is not sufficiently sensitive as a biomarker in drug development programs for clinically significant drug-related renal injury. This presents a problem for the monitoring of clinical trial subjects when they are exposed to potentially nephrotoxic agents. In addition to the sensitivity issue, the long delay that often occurs between an acute kidney injury event and a detectable rise of serum creatinine levels is problematic. To address these limitations, a number of other serum or urine biomarkers for acute kidney injury which I have listed here are being developed. Some of these biomarkers appear to have the advantage of increased sensitivity for early renal toxicity. In addition, combinations of these may enable discrimination between various forms of tubular and glomerular drug-related toxicity.

Studies of Pharmacogenomic & HLA Markers of DILI Susceptibility to Specific Agents

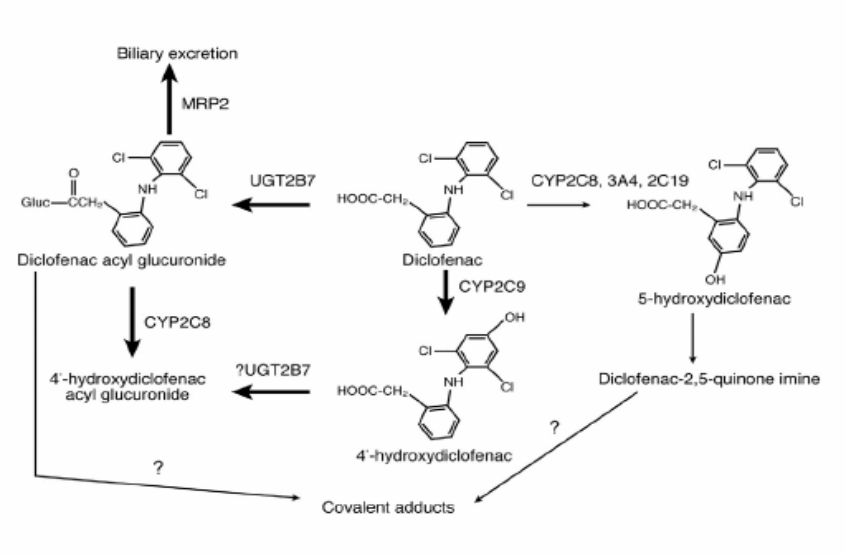
Examples

- ***Diclofenac***
- ***Amoxicillin/Clavulanic Acid***
- ***Ximelagatran***

Before finishing today, I want to touch on three examples of drugs for which pharmacogenomic or HLA markers have been associated with idiosyncratic DILI in the published literature, in order to highlight some of the investigational approaches that have been taken and the challenges that still need to be addressed for the successful development of clinically useful biomarkers that predict DILI.

Diclofenac-Associated Hepatotoxicity

Drug Structure & Metabolism



From Daly et al., Gastro. 2007

Like structurally related NSAIDs, diclofenac has a phenylacetic acid group that is linked by an amino bridge to an aryl halide moiety. A number of the 'fenac' drugs with these structural features have been associated with idiosyncratic hepatotoxicity. One explanation for this is that parent drugs in this class can be metabolized into electrophilic intermediates through a variety of pathways which then form adducts with cellular proteins, As has been published by Ann Daly and her colleagues, in this scheme 5-hydroxylation of diclofenac leads to a potential reactive product. In addition, glucuronidation through UGT2B7 produces a conjugate which is inherently unstable and capable of forming adduct structures when there is slowing in its transfer to bile because of reduced activity of the canalicular membrane transporter protein - MRP2. Using this scheme one can imagine that in some instances genetic variants with altered enzymatic activities which are responsible for each of these steps would cause build-up of some of these reactive metabolites, leading to an increased risk for idiosyncratic DILI.

Diclofenac-Associated Hepatotoxicity
Genetic Susceptibility

- **UK case control study: Daly et al. (Gastro., 132, 272-281, 2007)**
  - **DILI patients: 24 Northern European pts with diclofenac associated liver injury (79% female; 6 jaundice, 3 liver failure, 15 raised liver enzymes)**
  - **Controls: 48 diclofenac treated individuals without DILI**
  - **Enzymic activities of tested genetic variants vs wild-type: UGT2B7*2: high; CYP2C8*4: mixed; ABCC2 C-24T: reduced?**
  - **UGT2B7*2 frequency: 96% DILI Cases vs 73% Controls (OR ~ 8.5, 7.7; p = 0.3)**
  - **CYP2C8*4 frequency: 29% Cases vs 10% Controls (OR ~ 3.5; p = 0.09)**
  - **ABCC2 C-24T frequency: 70% Cases vs 39% Controls (OR 5.0; p = 0.005)**
  - **21% of all DILI cases both homozygous for UGT2B72* & heterozygous for CYP2C8*4 vs 2% Controls (OR ~ 12.4)**
  - **Results similar with 112 healthy community based control subjects**

Guided by this model, the Newcastle Group in the UK has employed a case control method to compare the frequencies of certain allelic variants of these enzymatic or transport activities in referred patients with diclofenac-induced hepatotoxicity compared with a matched control group of 'tolerators'. Using this approach the investigators found that each of these variants was associated with a higher frequency of DILI compared to the controls. With some combinations of these variants the statistical association with heightened frequency of diclofenac-induced liver injury was further strengthened.

Diclofenac-Associated Hepatotoxicity

Assessment of Genetic Risk

- **'At risk' genotypes of UGT2B7, CYP2C8 & ABCC2 may be complemented by 'at risk' IL-10 (lower activity) & IL-4 (higher activity) genotypes?***
- **Difference in the association of markers with mild vs severe liver injury not demonstrated**
- **Rate of clinically serious DILI from diclofenac < 1/10,000**
- **High prevalence in Community Controls of UGT2B7*2 (75%), CYP2C8*4 (17%), & ABCC2 C-24T (28%) leads to very low pos. predictive value of each 'at risk' biomarker or various combinations**
- **Compared to single 'at risk' biomarkers, combinations may modestly enhance specificity but are often accompanied by lower sensitivity**
- **Theoretically, testing must be performed on > 10,000 subjects to prevent 1 case of DILI**
- **Current results provide basis for ongoing research and are 'exploratory'**

*** Aithal, G.P. et al., Hepatology 39, 1431, 2004**

The Newcastle group has raised the interesting possibility that an increase in DILI risk caused by genetic alterations of diclofenac metabolizing enzymes or transporters may be complemented by genetically altered cytokine pathways that are involved in T cell recruitment and inflammatory responses, since in separate studies genomic variants with altered Il-10 and Il-4 activities have also been found to be associated with a higher frequency of liver injuries caused by the NSAID. Even if these findings are corroborated by others, testing for these pharmacogenomic variants as biomarkers to guide clinical practice decisions would be problematic. Clinically serious DILI outcomes after diclofenac exposure are extremely rare – in the order of 1 in 10,000. Since the genetic variants with altered enzymatic activities that I mentioned have relatively high prevalence in the population, it follows that even those individuals who would test positive would have a very small chance of developing DILI if treated with diclofenac. What we would end up with is a set of tests that are limited because of their very low positive predictive value. There would be a need to test thousands of patients to prevent even one case of diclofenac induced DILI. So to be used as a set of clinical tools to guide patient care we're not where we need to be.

HLA association with DILI
Amoxicillin – Clavulanate

– **Belgian Study: Hautekeete et al. (Gastro: 117; 1181-1186; 1999)**
- **35 pts with biopsy documented DILI**
- **Higher frequency of DRB1*1501-DRB5*0101-DQB1*0602 haplotype compared to BM donor controls (57% vs 12%)**
- **cholestatic or mixed pattern phenotype**
- **haplotype associated with extra-hepatic manifestations of hypersensitivity**

– **Scottish Study: O'Donohue et al. (Gut: 47; 717-720; 2000)**
- **22 pts with causally associated DILI (RUCAM)**
- **Higher frequency of DRB1*1501-DRB5*0101-DQA1*0102-DQB1*0602 haplotype compared to racially matched healthcare workers 70% (14/20) vs 20% (27/134) OR = 9; Homozygous for haplotype: 35% vs 1.5%; OR = 3**
- **Heterozygotes vs homozygotes: No difference in DILI manifestations**

– **Assessment: exploratory research; very low positive predictive value & limited sensitivity in study populations**

Another example of pharmacogenomic marker studies that have identified a biomarker association with DILI are the investigations of HLA variants in patients with hepatotoxicity caused by amoxicillin-clavulanate. Both of these studies have demonstrated that Northern Europeans who developed cholestatic or mixed forms of liver injury as a result of treatment with this agent have a higher frequency of the specific class II HLA allele with the tightly linked specific DRB1, DQA1 and DQB1 gene variants that are shown in this slide, compared to demographically matched controls. As in the case of diclofenac associated DILI, the rarity of serious liver injury from amoxicillin-clavulanic acid precludes use of pharmacogenomic testing of this locus alone as a useful clinical tool since the finding of the specific allele in Northern Europeans - even in its homozygous form - has both very low positive predictive value and limited sensitivity as a biomarker for DILI susceptibility.

Ximelagatran-Associated Hepatotoxicity

Genetic Susceptibility

Sponsor case control study (Kindmark A. et al., Pharmacogenomics J., 2007)

- **Retrospective exploratory study (EXGEN) of genetic samples collected during sponsor clinical studies;**
- **Study Methods: SNP analyses using both Genome Wide Scan (GWS) & Targeted Gene Analysis (TGA) of 690 candidate genes**
  - **74 pts: ALT ≥ 3xULN during treatment (liver injury)**
  - **39 pts: ALT > 1xULN & < 3xULN ('intermediate controls')**
  - **130 pts: ALT ≤ 1xULN (controls)**
- **Replication study**
  - **10 pts: ALT ≥ 4xULN during treatment (liver injury)**
  - **16 pts: ALT ≤ 1xULN (controls)**

And finally, with the marketing of ximelagatran having been terminated because of its association with clinically serious cases of DILI, a study was performed using samples from patients enrolled in the sponsor's clinical development program to identify possible pharmacogenomic DILI susceptibility biomarkers. Unfortunately, only a limited number of samples were available from study subjects who developed liver injuries which were mild and self-limited as well as ximelagatran treated controls who did not develop hepatotoxicity. Another limitation was that samples from patients who developed more serious forms of liver injury linked to ximelagatran treatment in the drug development program were not available in either the exploratory study or a subsequent very small replication study. With the samples that were available, the investigators sought to identify SNP genomic linkage markers, using both a genome wide scan approach as well as targeted analysis at the loci of only 690 specific candidate genes known to be transcriptionally active in the presence of hepatocellular injury or treatment with ximelagatran.

Ximelagatran-Associated Hepatotoxicity

Genetic Susceptibility & Study Results

Sponsor case control study (Kindmark A. et al., Pharmacogenomics J., 2007)

- **Case samples primarily from patients with mild liver injury**
- **GWS of 266,000 SNPs: All associations with liver enzyme elevations were weak due to statistical adjustments for multiple testing**
- **TGA: Statistically significant HLA associations with DRB1*07 & DQA1*02, both in exploratory and replication studies.**
- **Other gene polymorphism associations identified in the exploratory study were not confirmed in replication study. Cannot distinguish between low power & false positives.**
- **Frequency of DRB1*07 in pts with liver enzyme elevations vs controls: 26% vs 8.5%**
- **Limited sensitivity & specificity**

Because of powering limitations, these researchers were severely handicapped by the small size of the test sample sets, both in the exploratory and replication studies.

Only the targeted gene analysis was able to yield two statistically significant HLA class II allelic associations which were confirmed in the replication study. Generally, it was not possible to determine whether most of the pharmacogenomic associations found in the exploratory study were the result of chance alone or were real, but not reproducible, because of under-powering in the replication study. In retrospect, because of random statistical variation that must be discounted when using a genome wide scan approach, much higher powering would have been required in order to overcome the false discovery background effect. From this limited investigation of ximelagatran, the HLA associations that were uncovered are not strong and characterized by both limited sensitivity and specificity for idiosyncratic susceptibility to ximelagatran-induced liver injury.

Ximelagatran-Associated Hepatotoxicity

Genetic Susceptibility & Lessons Learned

- **Optimal study elements should include:**
  - **Systematic collection of samples from all clinical trial subjects (DILI & control pts in exploratory & replication studies) to gain statistical power**
  - **Samples from pts with severe DILI as well as mild liver injury**
  - **Regular liver test monitoring during treatment & documentation of pt phenotypes**
- **Possible barriers in biomarker discovery include:**
  - **More than one independent genotype linked to DILI, each with low sensitivity**
  - **Single genetic changes necessary but not sufficient to cause severe DILI (each has low specificity)**
  - **Technical difficulties in genotyping**
  - **Complementary metabonomic studies may enhance predictive power**

An important take home lesson from this ximelagatran study is that systematic prospective collection of genomic samples must be performed on all clinical trial subjects, including those receiving the test medication who develop drug-related toxicity - the 'susceptibles' and 'adaptors' - as well as all 'tolerators' and placebo control subjects. In addition, patient phenotypes must be well documented and reliably ascertained through consistently applied liver monitoring practices during all phases of treatment. When a drug-related liver problem emerges during clinical trials, such a meticulously developed database might be an invaluable tool to help develop an approach to understand and manage the risk.

Candidate DILI Biomarkers

Points for Consideration

So far, low positive predictive value of 'sensitive' liver enzyme markers for clinically significant DILI!

Marker(s) that predict clinically significant liver injury

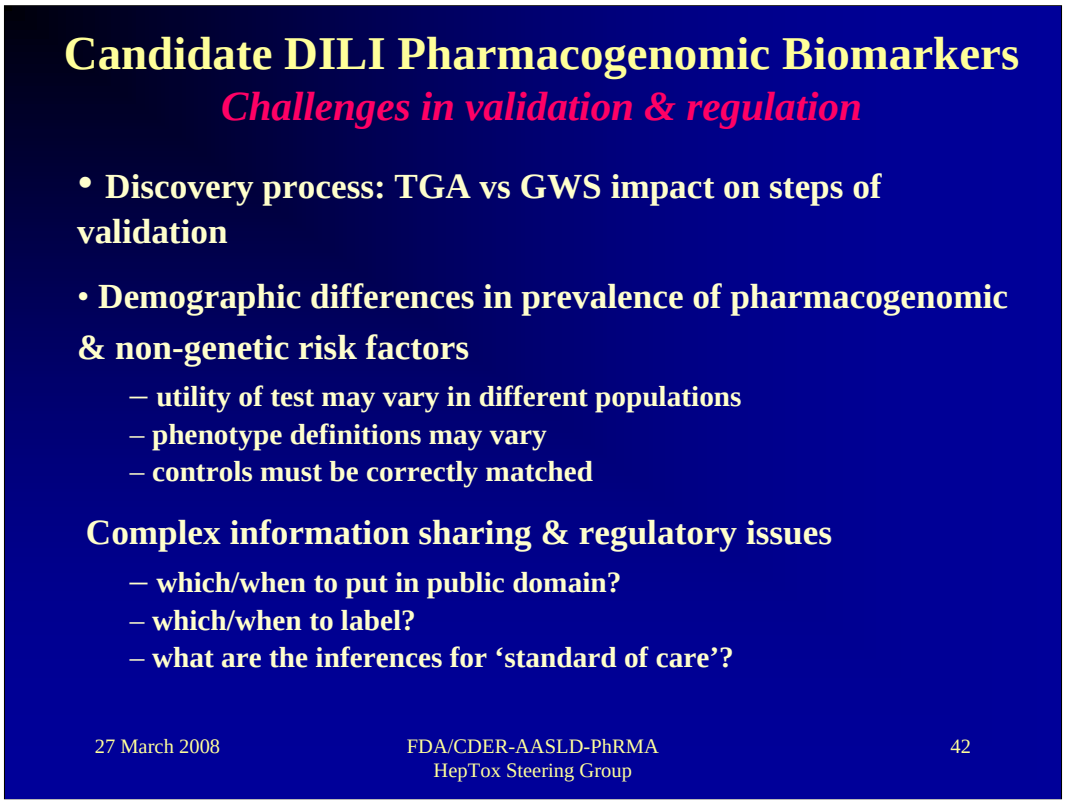
- **Sensitivity**
  - **– In clinical trials what was monitoring adherence and frequency?**
- **Specificity**
  - **– Does marker**
    - **discriminate from liver injury that will be mild & transient?**
    - **discriminate from other sites of injury?**
    - **discriminate from non-drug etiologies?**

Pharmacogenomic/HLA marker(s) of individual susceptibility

- **What is prevalence of marker in population vs incidence of toxicity?**
- **If many false positives is there an alternative treatment(s) in marker pos pts?**

In conclusion, so far, candidate DILI biomarkers that have recently been identified can be characterized as having limited predictive value for progression to clinically serious hepatotoxicity. The need for specific biomarkers that discriminate cases which are destined to progress to clinically significant drug-induced liver injury cannot be understated.

In the future, utility of new pharmacogenomic biomarkers of susceptibility to DILI will be heavily influenced by their background prevalence in a demographically matched population and the range of clinical outcomes from liver injury that would be prevented.



Finally, after a exploratory study of a new pharmacogemomic biomarker is completed, the type of validation study that could be considered might be affected by the method used in the exploratory study. If a genome wide scan has been used during an initial study, because of the high false discovery rate based on multiplicity of loci tested, it is critical to exhaustively rule out random artifactual associations in follow-up studies. In the case that targeted gene analysis has been used for discovery, although hypothesis driven, the possibility that stronger biomarker associations with DILI exist at untested loci should be considered during the planning of follow-up studies.

In characterizing the utility of a pharmacogenomic biomarker for DILI, there may be important demographic influences to consider. These include the relative contribution of the specific genetic locus with other factors on risk for DILI, the phenotype of liver injury that is observed and the demographic prevalence of the genetic variant or allele that is being investigated as a biomarker.

Also, there are complex information sharing and regulatory issues for FDA to consider with regards to how physicians and patients should be instructed about the use of new DILI biomarkers. These include which types of data should be put into the public domain, what criteria should be applied when deciding to incorporate DILI biomarker data into product labeling to instruct patient risk management and what inferences would be expected by FDA communications or labeling on DILI biomarkers on the 'standard of care' of patients.

Summary

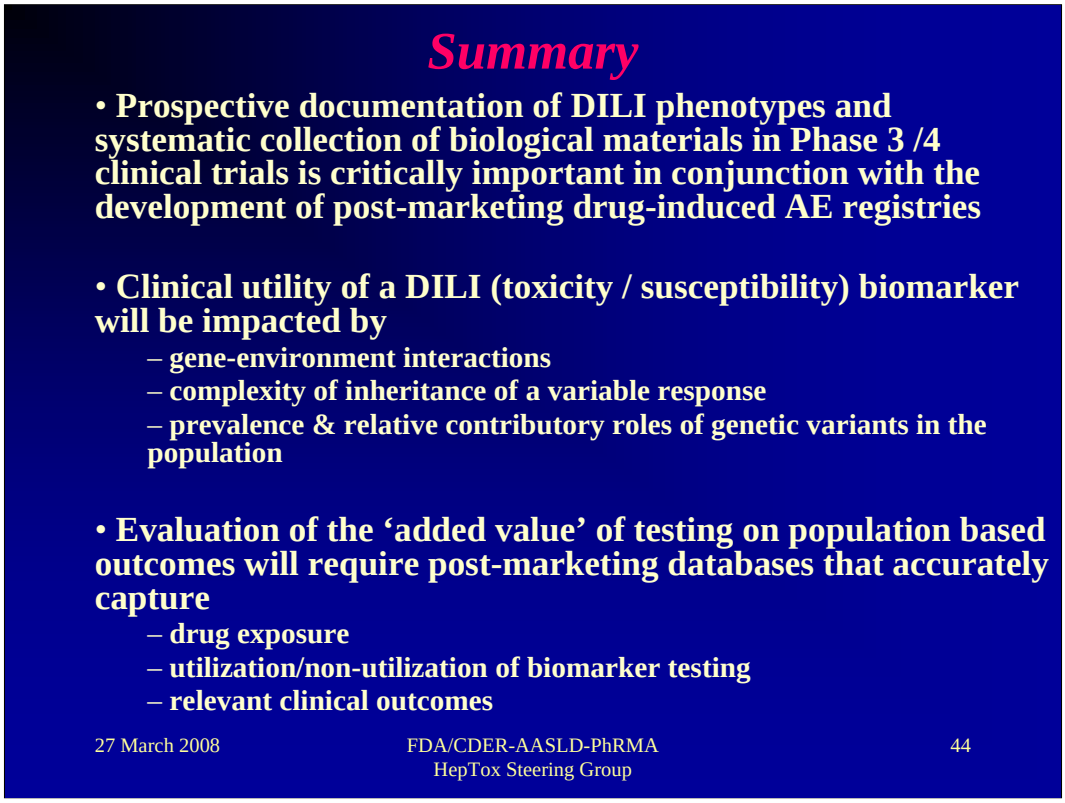
- **Predictive measurements needed for early drug development decisions vs risk management of patients may not rely on the same set(s) of biomarkers**

- **Pharmacogenomic biomarkers that predict idiosyncratic DILI with adequate sensitivity & positive predictive value for clinical use await discovery**

The discovery of biomarkers as predictors of clinically significant DILI (toxicity / susceptibility) after marketing depends on

- **– validation using appropriate matched controls**
- **– rigorous clinical correlation with defined phenotypes**
  - **eg. 'susceptible', 'tolerator', 'adaptors', etc.**
- **– comprehensive risk evaluation**

In summarizing my talk there are a few key points to remember - and I have listed them. Among these points, pharmacogenomic biomarkers that predict idiosyncratic DILI with adequate sensitivity and positive predictive value for clinical use await discovery. So they need to be discovered! This will require a robust scientific effort by industry, academia and governmental groups.



Another key take home message is that meticulous prospective documentation of DILI events and systematic collection of biological materials from all enrolled study subjects in clinical trials is critically important. Data from clinical trials might be complemented with pharmacogenomic and other biomarker information that could be gathered from patients enrolled in post-marketing drug-induced AE registries.

Finally, evaluation of the 'added value' of testing on population-based outcomes will require post-marketing databases that accurately capture drug exposure, utilization or non-utilization of biomarker testing and relevant clinical outcomes.

Conclusion

Ongoing discovery and validation of DILI biomarkers as predictors of serious toxicity or individual susceptibility to liver injury throughout the lifecycle of certain drugs is inevitable. To reflect new findings that may impact on optimal risk management in a genetically diverse population, continuing review of biomarker data and cumulative refinement of drug labeling may be necessary at different points in the post-marketing phase. It is important to systematically evaluate and effectively communicate any new information on biomarker testing that is validated & predicted to enhance patient care.

And lastly, to express the essence of the statement on this slide - It is inevitable that research on DILI biomarkers pertinent to a specific drug will continuously expand both before and after its marketing. The process for critically analyzing, updating and contextualizing complex growing biomarker datasets, the development of optimal information tools for storing and communicating review findings and the establishment of consistent criteria for refining product labeling at different points in the post-marketing phase are exciting challenges for FDA in this new arena that must be addressed.

I'm going to end there.

(Applause.)