Pharmacogenetic of Drug Hypersensitivity:
Personalising Medicine & Predicting the Unpredictable

Elizabeth J. Phillips, MD, FRCPC
Professor & Director, Centre for Clinical Pharmacology & Infectious Diseases, Murdoch University
Royal Perth Hospital, Sir Charles Gairdner Hospital
Perth, Western Australia

WAMSG 5th Annual Medication Safety Symposium 14 July 2009
Genetics & Adverse Drug Reactions

“If it were not for the great variability among individuals, medicine might as well be a science and not an art”

Sir William Osler, 1892
Personalizing Medicine = defining optimal diagnostic, management & treatment strategies for the individual patient

**Pharmacogenetics**
- Specific tests to prevent toxicity (eg. HLA-B*5701 screening for abacavir hypersensitivity)
- Guide dosing of drugs
- HLA and other immune markers to guide vaccine development and predict response

**Pharmacoeecology**
- Individualizing the patient’s environment to optimize therapeutic outcome
- PK/PD interactions with drugs, herbal medicines and nutritional supplements
- Food effects on drugs (absorption/bioavailability)
- Adherence (cultural & socioeconomic influences)
- Intercurrent disease of host state (eg organ failure, pregnancy)
- Diurnal variation in pharmacokinetics/dynamics
- Individual drug taking behaviour

**Personalized Prescription**

*Phillips E, Mallal S* Personalized Medicine 2009 (in press)
### Characteristics of Drug Reactions

<table>
<thead>
<tr>
<th>Classification</th>
<th>TYPE A = Pharmacological</th>
<th>TYPE B = “Bizarre”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predictable</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dose Dependent</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Host Dependent (Genetic factors)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Immunologic basis</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Examples</td>
<td>Gastrointestinal intolerance, seizures with penicillin etc</td>
<td>Allergic and idiosyncratic syndromes, hypersensitivity syndromes</td>
</tr>
</tbody>
</table>
Classification of ADRs

Drug Reaction

Predictable Reactions (dose dependent)

Idiosyncratic
- Reactive
- Metabolite
- Syndromes (e.g., Drug Hypersensitivity Syndrome)
- Hepatitis
- SJS/TEN

Unpredictable Reactions (less dose dependent)

Hypersensitivity/
Immunogenetic
- Type I
- Type II
- Type III
- Type IVa-d

Pseudoallergic
- ASA
- Vancomycin
- Contrast
TYPE B REACTIONS: Associated Drugs

- **Anticonvulsants**
  - phenytoin, phenobarbital, carbamazepine
  - lamotrigine
- **Antimicrobials**
  - sulfonamides (sulfa antimicrobials), penicillins, dapsone
  - nitrofurantoin, minocycline, metronidazole
- **Allopurinol**
- **Antiinflammatories** (eg oxicam-NSAIDS, Valdecoxb)
- **Antiretrovirals**
- **Alternative medicines**
  - Chinese herbals etc.
- **Antineoplastics**
- **mAbs**
  - “Ximab” (infliximab, rituximab) > “Zumab” (omalizumab) > “Mumab” (adalimumab)
Pharmacogenetics

Drug → Disease → Gene

TYPE A

Dose Dependent Disease

- Drug Efficacy or toxicity
- Often multifactorial (e.g., age, gender, BMI, concurrent medications)
- Often related to polymorphism(s) in drug metabolizing enzymes (e.g., CYP) or pharmacodynamic factors
- e.g., Warfarin, efavirenz, clopidogrel

TYPE B

Dose Independent Disease

- HLA & Drug Hypersensitivity
  1. abacavir hypersensitivity (HLA-B*5701)
  2. carbamazepine SJS/TEN (HLA-B*1502)
  3. phenytoin SJS/TEN (HLA-B*1502)
  4. allopurinol SJS/TEN/HSR (HLA-B*5801)
  5. ximelagatran hepatotoxicity (HLA-DRB1*07; DQA1*02)

  5. nevirapine rash and rash associated hepatitis (class I (HLA-DRB1*0101 + CD4+, HLA-B*1402, HLA-Cw8, HLA-B*3505))

   Locharenkul et al. Epilepsia 2008;49:2087-91
4. Kindmark et al. Pharmacogenomics J 2008;8:186-95
HLA & Drug Hypersensitivity

Lancet March 2, 2002

Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir

F. T. Christiansen

Summary

Background: The use of abacavir—a potent HIV-1 nucleoside-analogue reverse-transcriptase inhibitor—is complicated by a potentially life-threatening hypersensitivity syndrome in about 5% of cases. Genetic factors influencing the immune response to abacavir might confer susceptibility. We aimed to find associations between MHC alleles and abacavir hypersensitivity.

Interpretation: Genetic susceptibility to abacavir hypersensitivity is carried on the 57-1 ancestral haplotype. In our population, withholding abacavir in those with HLA-B*5701, HLA-DR7, and HLA-DQ3 should reduce the prevalence of hypersensitivity from 5% to 2.5% without inappropriately denying abacavir to any patient.

Lancet March 30, 2002

Genetic variations in HLA-B region and hypersensitivity reactions to abacavir


Hypersensitivity to abacavir affects about 4% of patients who receive the drug for HIV-1 infection. We did a retrospective, case-control study of 113 patients with evidence of hypersensitivity to abacavir and 158 controls. HLA-B*5701 occurred more frequently among patients than controls by Fisher's exact test. These included HLA-B*5701 (9/158) of 6% controls vs 24/113 (21%) of patients (P=0.002).
A marker for Stevens-Johnson syndrome

Stevens-Johnson syndrome and the related disease toxic epidermal necrolysis are life-threatening reactions of the skin to particular types of medication. Here we show that there is a strong association in Han Chinese between a genetic marker, the human leukocyte antigen HLA-B*1502, and Stevens-Johnson syndrome induced by carbamazepine, a drug commonly prescribed for the treatment of seizures. It should be possi-

### Table 1 Frequency of HLA alleles in patients with Stevens-Johnson syndrome

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>CEB-SJS</th>
<th>CEB-Tolent</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*1502</td>
<td>44 (100%)</td>
<td>3 (6%)</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>Cw*0301</td>
<td>41 (83.2%)</td>
<td>17 (33.8%)</td>
<td>13 (14%)</td>
</tr>
<tr>
<td>A*1101</td>
<td>36 (81.8%)</td>
<td>61 (60.6%)</td>
<td>63 (67%)</td>
</tr>
<tr>
<td>DRB1*1502</td>
<td>33 (73%)</td>
<td>12 (13%)</td>
<td>18 (19.4%)</td>
</tr>
<tr>
<td>B<em>1502, Cw</em>0301</td>
<td>41 (83.2%)</td>
<td>3 (6%)</td>
<td>7 (7.5%)</td>
</tr>
<tr>
<td>B<em>1502, A</em>1101</td>
<td>36 (81.8%)</td>
<td>2 (2%)</td>
<td>6 (6.5%)</td>
</tr>
<tr>
<td>B<em>1502, DPE1</em>1202</td>
<td>33 (73%)</td>
<td>1 (1%)</td>
<td>5 (5.4%)</td>
</tr>
<tr>
<td>B<em>1502, Cw</em>0301, A<em>1101, DPE1</em>1202</td>
<td>26 (60%)</td>
<td>0 (0%)</td>
<td>3 (3.2%)</td>
</tr>
</tbody>
</table>

Frequencies, by number and percentage, of individuals or combined sets of the B*1502 allele and haplotype are shown in patients with carbamazepine-induced Stevens-Johnson syndrome (CEB-SJS; n = 44), and in carbamazepine-tolerant (n = 101) and normal subjects (n = 99). For methods, see supplementary information.

Odds ratio (CEB-SJS vs CEB-Tolerant): 2.38 (95% CI: 1.23–4.63, 3); corrected P = 3.13 x 10^-4.

Fisher exact test (CEB-SJS vs Normal): 696 (1506) 0.05-15.69; P = 1.98 x 10^-4.

---

**WARNING**

SERIOUS DERMATOLOGIC REACTIONS AND HLA-B*1502 ALLELE

Serious and sometimes fatal dermatologic reactions, including toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS), have been reported during treatment with tegretol. These reactions are estimated to occur in 1 to 6 per 10,000 new users in countries with mainly Caucasian populations, but the risk in some Asian countries is estimated to be about 10 times higher. Studies in patients of Chinese ancestry have found a strong association between the risk of developing SJS/TEN and the presence of HLA-B*1502, an inherited allelic variant of the HLA-B gene. HLA-B*1502 is found almost exclusively in patients with ancestry across broad areas of Asia. Patients with ancestry in genetically at-risk populations should be screened for the presence of HLA-B*1502 prior to initiating treatment with tegretol. Patients testing positive for the allele should not be treated with tegretol unless the benefit clearly outweighs the risk (see warnings and precautions/laboratory tests).
WARNING

SERIOUS DERMATOLOGIC REACTIONS AND HLA-B*1502 ALLELE

SERIOUS AND SOMETIMES FATAL DERMATOLOGIC REACTIONS, INCLUDING TOXIC EPIDERMAL NECROLYSIS (TEN) AND STEVENS-JOHNSON SYNDROME (SJS), HAVE BEEN REPORTED DURING TREATMENT WITH TEGRETOL. THESE REACTIONS ARE ESTIMATED TO OCCUR IN 1 TO 6 PER 10,000 NEW USERS IN COUNTRIES WITH MAINLY CAUCASIAN POPULATIONS, BUT THE RISK IN SOME ASIAN COUNTRIES IS ESTIMATED TO BE ABOUT 10 TIMES HIGHER. STUDIES IN PATIENTS OF CHINESE ANCESTRY HAVE FOUND A STRONG ASSOCIATION BETWEEN THE RISK OF DEVELOPING SJS/TEN AND THE PRESENCE OF HLA-B*1502, AN INHERITED ALLELIC VARIANT OF THE HLA-B GENE. HLA-B*1502 IS FOUND ALMOST EXCLUSIVELY IN PATIENTS WITH ANCESTRY ACROSS BROAD AREAS OF ASIA. PATIENTS WITH ANCESTRY IN GENETICALLY AT-RISK POPULATIONS SHOULD BE SCREENED FOR THE PRESENCE OF HLA-B*1502 PRIOR TO INITIATING TREATMENT WITH TEGRETOL. PATIENTS TESTING POSITIVE FOR THE ALLELE SHOULD NOT BE TREATED WITH TEGRETOL UNLESS THE BENEFIT CLEARLY OUTWEIGHS THE RISK (SEE WARNINGS AND PRECAUTIONS/LABORATORY TESTS).
HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol

Shuen-Iu Hung\textsuperscript{a,b}, Wen-Hung Chung\textsuperscript{a,b,c,d}, Lieh-Bang Liou\textsuperscript{e}, Chen-Chung Chu\textsuperscript{f}, Marie Lin\textsuperscript{g}, Hsien-Ping Huang\textsuperscript{a}, Yen-Ling Lin\textsuperscript{a}, Joung-Liang Lan\textsuperscript{h}, Li-Cheng Yang\textsuperscript{i}, Hong-Shang Hong\textsuperscript{j}, Ming-Jing Chen\textsuperscript{k}, Ping-Chin Lai\textsuperscript{l}, Mai-Szu Wu\textsuperscript{m}, Chia-Yu Chu\textsuperscript{a}, Kuo-Hsien Wang\textsuperscript{a}, Chien-Hsiun Chen\textsuperscript{a}, Cathy S. J. Fann\textsuperscript{a}, Jer-Yuarn Wu\textsuperscript{a}, and Yuan-Tsong Chen\textsuperscript{a}\textsuperscript{m}

Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan; Departments of \textsuperscript{b}Dermatology, \textsuperscript{d}Rheumatology, Allergy, and Immunology, and \textsuperscript{e}Nephrology, Chang Gung Memorial Hospital, Taipei 11057, Taiwan; \textsuperscript{f}Department of Medical Research, Mackay Memorial Hospital, Taipei 10449, Taiwan; \textsuperscript{g}Department of Immunology and Rheumatology, Taichung Veterans General Hospital, Taichung 40701, Taiwan; \textsuperscript{h}Department of Dermatology, National Taiwan University Hospital, Taipei 10062, Taiwan; \textsuperscript{i}Department of Dermatology, Taipei Medical University Hospital, Taipei 11031, Taiwan; \textsuperscript{k}Department of Medical Research, China Medical University Hospital, Taichung 40447, Taiwan; \textsuperscript{l}Department of Pediatrics, Duke University Medical Center, Durham, NC 27710; and \textsuperscript{m}Molecular Medicine Program, Taiwan International Graduate Program, Academia Sinica and the School of Life Sciences, National Yang Ming University, Taipei 11529, Taiwan

LETTERS

published online May 31 2009

HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin

Ann K Daly\textsuperscript{1}, Peter T Donaldson\textsuperscript{1}, Pallav Bhatnagar\textsuperscript{1}, Yufeng Shen\textsuperscript{2}, Itaile Pe'er\textsuperscript{3}, Aris Horanos\textsuperscript{4}, Mark J Daly\textsuperscript{5}, David B Goldstein\textsuperscript{6}, Sally John\textsuperscript{7}, Matthew R Nelson\textsuperscript{8}, Julia Graham\textsuperscript{3}, B Kevin Park\textsuperscript{9}, John F Dillon\textsuperscript{10}, William Bernal\textsuperscript{10}, Heather J Cordell\textsuperscript{10}, Munir Pirmohamed\textsuperscript{10}, Gunprasad P Aithal\textsuperscript{10,11} & Christopher P Day\textsuperscript{1,11}, for the DILIGEN study\textsuperscript{2} and International SAE Consortium\textsuperscript{23}
Associations may differ across race, phenotype, study design and underlying immune status

- HLA-DRB1*0101 + CD4% > 25 and rash associated hepatitis (Martin et al AIDS 2005)
- HLA-Cw8-B14 haplotype in Sardinians (Littera et al AIDS 2006)
- HLA-Cw8 in Japanese (Gatanaga et al AIDS 2007)
- HLA-DRB1*01 in French population with efavirenz or nevirapine rash

**Pharmacogenetics & Genomics 2009**

**HLA-B*3505** allele is a strong predictor for nevirapine-induced skin adverse drug reactions in HIV-infected Thai patients

Soranun Chantarangau, Taisei Mushiroda, Surakameth Mahasirimongkol, Sasisopin Kiertiburanakul, Somnuek Sungkanuparph, Weerawat Manosuthi, Woraphot Tantisiriwat, Angkana Charoenyingwattana, Thanyachai Sura, Wasun Chanratitita and Yusuke Nakamura
NEVIRAPINE HYPERSENSITIVITY (rash of varying severity, fever, hepatitis, multisystem disease)

- Genetic susceptibility conferred by Class I MHC marker(s) eg. HLA-B*1402, HLA-Cw8, HLA-B*3505
- Genetic susceptibility conferred by Class II MHC marker(s) eg. HLA-DRB1*0101 + Sufficient levels of CD4+ T cells
  - Sufficient levels of relevant metabolite (distinct from dose dependent or “Type A” adverse drug reactions)
    - Nevirapine disposition (?Role of CYP3A4/CYP2B6/CYP2C9) and drug transporters (P-glycoprotein(MDR1)) and polymorphisms in drug metabolizing and drug transporter genes that differ across ethnicity

Phillips E, Mallal S. Current Opinion in Molecular Therapeutics 2009; 11(3):231-242
Host-Drug-Infection Interactions: A Complex Dynamic

HOST

- HLA
- Immune response genes
- Control of infection
- Vaccine design

PATHOGEN

- Chronic persistent DNA viruses (CMV, EBV, HHV-6, Herpes viruses)
- HIV

DRUG

- Pharmacodynamics
- Pharmacokinetics
- Drug Efficacy
- Drug Toxicity
- Drug resistance
- Drug Efficacy
Classic 3 ring Target – HSV not drug

Atypical Target – 2 ring dusky center

Mucositis & Atypical target

Blistering of TEN
HLA-B*1502 & Carbamazepine: Phenotype is Important

Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis

-Associated with severe skin syndromes (SJS/TEN in Han Chinese)

-Not associated with drug hypersensitivity (fever/rash/internal organ involvement) or disease in Caucasians
Prerequisites for Widespread Incorporation of HLA Pharmacogenetic Testing

Implications differ according to drug…
<table>
<thead>
<tr>
<th>Prerequisites</th>
<th>Drug/HLA association</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test</strong></td>
<td>ABC</td>
</tr>
<tr>
<td>• HLA allele is strongly associated with the toxicity, and the negative predictive value of the test is high*</td>
<td>+++</td>
</tr>
<tr>
<td>• The number of patients needed for testing to prevent a case of toxicity is low*</td>
<td>++</td>
</tr>
<tr>
<td>• HLA allele is prevalent in a large, non-disenfranchised population*</td>
<td>++</td>
</tr>
<tr>
<td><strong>Drug</strong></td>
<td></td>
</tr>
<tr>
<td>• Drug exhibits favorable attributes, such as good efficacy, convenience in dosing and administration, tolerability and pill burden*</td>
<td>++</td>
</tr>
<tr>
<td>• Alternative drug(s) that do not require pharmacogenetic testing are either absent or have negative attributes*</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Drug toxicity</strong></td>
<td></td>
</tr>
<tr>
<td>• Toxicity is severe and persistent* (ie, not isolated mild rash)</td>
<td>++</td>
</tr>
<tr>
<td>• Toxicity is readily and accurately phenotyped*</td>
<td>++</td>
</tr>
<tr>
<td>• An adjunctive diagnostic test, such as skin patch testing, can improve phenotypic precision</td>
<td>++</td>
</tr>
<tr>
<td><strong>Environment</strong></td>
<td></td>
</tr>
<tr>
<td>• Champions available (eg, clinical academics, industry [if drug not off patent*], professional bodies, regulatory agencies, guideline committees, patient advocacy groups, laboratory providers and the media), willing and able to drive pharmacogenetic test development and implementation</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Generation of high-level evidence</strong></td>
<td></td>
</tr>
<tr>
<td>• Case-control studies with estimated predictive values based on the assumed prevalence of the HLA allele</td>
<td>++</td>
</tr>
<tr>
<td>• Population-based cohort studies with directly calculated predictive values of the test</td>
<td>++</td>
</tr>
<tr>
<td>• Open screening studies</td>
<td>++</td>
</tr>
<tr>
<td>• Supportive experimental data</td>
<td>++</td>
</tr>
<tr>
<td>• Blinded randomized controlled trials</td>
<td>+++</td>
</tr>
<tr>
<td>• Evidence across ethnic groups and geographical areas to determine the clinical settings that the test may be applied to</td>
<td>+++</td>
</tr>
<tr>
<td>• Cost-effectiveness data</td>
<td>++</td>
</tr>
<tr>
<td><strong>Development and availability of appropriate laboratory support</strong></td>
<td></td>
</tr>
<tr>
<td>• No patent restriction on use of the test</td>
<td>++</td>
</tr>
<tr>
<td>• Development of simple, inexpensive, robust, unambiguous laboratory tests</td>
<td>+</td>
</tr>
<tr>
<td>• Rapid and simple report and interpretation</td>
<td>++</td>
</tr>
<tr>
<td>• Development of reagents (eg, mAbs, PCR-based kits)</td>
<td>++</td>
</tr>
<tr>
<td>• Global distribution and commercialization of allele-specific test</td>
<td>++</td>
</tr>
<tr>
<td>• Allele-specific quality assurance targeted to avoid false-negative results and consequent morbidity or mortality</td>
<td>+</td>
</tr>
<tr>
<td>• Reimbursement of test</td>
<td>++</td>
</tr>
<tr>
<td><strong>Design and implementation of appropriate clinical systems</strong></td>
<td></td>
</tr>
<tr>
<td>• Education of clinicians, nurses, pharmacists, phlebotomists and patients</td>
<td>++</td>
</tr>
<tr>
<td>• Systems to ensure appropriate and routine triggering of ordering of the test</td>
<td>+</td>
</tr>
<tr>
<td>• Systems in the clinic to ensure the correct blood samples are sent to the correct laboratory for analysis</td>
<td>+</td>
</tr>
<tr>
<td>• Systems to ensure test results and correct interpretation is rapidly transmitted to, retained by and acted on by the healthcare team and patient</td>
<td>+</td>
</tr>
</tbody>
</table>
Factors Favouring Clinical Uptake of a Pharmacogenetic Test

- clinical outcome associated with the test has significant impact on patient well-being
- lack of alternative drug(s) with more favourable therapeutic/toxicity profile
- high predictive value of the genetic test
- ready availability of rapid, low cost test
- identification of clinical parameters that determine the usefulness of the test
- sufficient clinical utility to be incorporated into routine management, so that testing is performed prospectively without requiring specific ‘triggers’

Framework of Antiretroviral Therapy & Pharmacogenetics

- Define whether or not the drug should be used (dose independent toxicity)
  - Abacavir & HLA-B*5701 screening

- Define the optimal dose of the drug for a specific patient or patient population (dose dependent toxicity & efficacy)
  - NNRTIs, protease inhibitors

- Avoid drug/drug & drug gene interactions

- Define or enhance understanding of the pathophysiology of drug toxicity
  - Metabolic complications, lipodystrophy, peripheral neuropathy, mitochondrial toxicities
Translating Research in Genomic Medicine into the Clinic & Beyond

• **STEP 1 – The discovery & turning this into a health application**
  
  2002 – HLA-B*5701 association in two independent groups

  Imprecision of clinical phenotype (false positive clinical diagnosis) cast doubt on generalizability of HLA-B*5701 to “all” abacavir HSR. Patch testing developed to identify those with true immunologically medicated HSR

• **STEP 2 – Development of high level clinical evidence (randomized) in support of test. Basic science to support biological plausibility**

  2002-present: genetic and cellular studies support plausibility of association
  2005-2008 – PREDICT-1, SHAPE & observational studies provide robust clinical evidence

• **STEP 3 – Diffusion of research and delivery to clinic depends on efficiency and quality assurance of laboratory testing and reporting**

  2005 - PCR-based techniques readily applicable methodology (rapid, inexpensive)
  2005-2008 - HLA-B*5701 quality assurance program roll-out
  2008 – HIV Treatment guidelines/product information change to incorporate recommendation for baseline testing & reimbursement

• **STEP 4 – Evaluating performance of the test in real clinical practice**

  2008 + - Multiple observational studies Pharmacoeconomic evaluation
  Post “PREDICT-1” clinical quality assurance

Phillips, Mallal Personalized Medicine 2009 (in press)
Pharmacogenetics & Antiretroviral Therapy: The Abacavir Example

- Guanosine nucleoside analogue used in antiretroviral therapy, approved by regulatory agencies 1998/1999
- Hypersensitivity syndrome characterized by fever, malaise +/- skin rash was characterized in pre-marketing phase, not fatal unless drug reintroduced; affected approximately 6-8% of predominantly Caucasian population
- Robust clinical management program for drug
- Early clues such as familial case reports and rarity in Blacks suggested genetic association
- 2002 – two independent groups reported strong association with HLA-B*5701
- 2008 – screening for hypersensitivity has rolled out into much of developed world
**HLA-B*5701 and Abacavir Hypersensitivity: A Comparison of Two Studies**

<table>
<thead>
<tr>
<th>HSR</th>
<th>Pos</th>
<th>Neg</th>
<th>Sens</th>
<th>Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallal et al, 2002 HLA-B*5701</td>
<td></td>
<td></td>
<td>78%</td>
<td>97%</td>
</tr>
<tr>
<td>Pos PV</td>
<td>14</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg PV</td>
<td>5</td>
<td>177</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Hetherington et al, 2002* HLA-B57 |     |     | 55%   | 99%    |
| Pos PV       | 36  | 29  |       |        |
| Neg PV       | 8   | 649 |       |        |

* Caucasians only

Observed sensitivity and specificity

PV assumes prevalence of ~9%

Early Problems with "Phenotype"

- Early studies have observed variable sensitivity of HLA-B*5701
  - Definition of abacavir hypersensitivity reaction (ABC HSR)
    nonspecificity of clinical phenotyping  
    false positive clinical diagnosis
  - Differences in white and nonwhite races

![Bar chart showing sensitivity of HLA-B*5701](Mallal et al. Lancet 2002, Hughes et al. Pharmacogenomics 2004)
### Phenotypic Uncertainty of Abacavir HSR

<table>
<thead>
<tr>
<th>Blinded Study</th>
<th>Abacavir Groups</th>
<th>Zidovudine or indinavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNA3005</td>
<td>19/268 (7%)</td>
<td>6/265 (2%)</td>
</tr>
<tr>
<td>CNA30024</td>
<td>27/324 (8%)</td>
<td>10/325 (3%)</td>
</tr>
<tr>
<td>Total Cases</td>
<td>46/592 (7.8%)</td>
<td>16/590 (2.7%)</td>
</tr>
</tbody>
</table>

Cases in CNA30024 as reported by Investigators in the ABC HSR CRF Module

Patch Testing Following ABC Exposure

Preparing ABC ingestion

Sensitization

CYP450

ABC \rightarrow \text{Reactive Metabolite (Antigen)}

Conjugation with host protein in skin

Presentation by epidermal Langerhans cells

CD8+

Local Reaction

Day 0

>6 weeks later

Abacavir Skin Patch Testing

Patch testing

Previously assigned cases
Carriers of 57.1 AH markers 9/9
Non-57.1 carriers (NNRTI+) 0/3
Non-57.1 carriers (NNRTI-) 1 patient unavailable

Previously assigned tolerant
Carriers of 57.1 AH markers 0/5

3 previously assigned cases had diagnosis revised
(1) Concurrent NNRTI therapy
(2) Negative patch test
**HLA-B*5701 and Abacavir Hypersensitivity**

### HLA-B*5701

<table>
<thead>
<tr>
<th></th>
<th>Pos</th>
<th>Neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSR</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>No HSR</td>
<td>4</td>
<td>180</td>
</tr>
<tr>
<td>Pos PV</td>
<td>78.9%</td>
<td></td>
</tr>
<tr>
<td>Neg PV</td>
<td>99.4%</td>
<td></td>
</tr>
</tbody>
</table>

Sens 93.8%
Spec 97.8%

Reclassified first 200 patients (*not available*)

Incorporation of a Pharmacogenetic Test into Clinical Practice

- High level evidence generalisable to diverse clinical settings
- Widespread availability of cost-effective and reliable laboratory tests
- Effective strategies to operationalise testing into routine clinical practice
Possible Abacavir-related symptoms, number of patients (including definitive ABC-HSR)

Abacavir HSR, number of patients

# 2 pts results not reviewed prior therapy
$ 1 pt with informed choice/ incomplete haplotype

Proportion of ABC-naïve patients discontinuing ABC within 6 weeks

Before genetic screening

After genetic screening

1998/1999 n=68
2000/2001 n=131
2002/2003 n=107
2004 to 7/2005 n=60

*P<0.05

*Trottier et al  abacavir early discontinuation decreased from 13.6% to 5.6% after screening, IAS 2007, abstract MOPEB002)
+HLA-B*5701 + HSR from 12.2% to 0% (Zucman et al JAIDS 2007; epub March 8)
^ABC HSR 6.5% historically to 0% post screening (Reeves et al HIV Medicine March 2006)
*All 4/725 (0.8%) patients in ARIES study clinical diagnosed with HSR were patch test negative

HLA-B*5701 Screening for Hypersensitivity to Abacavir

Simon Mallal, M.B., B.S., Elizabeth Phillips, M.D., Giampaolo Carosi, M.D., Jean-Michel Molina, M.D., Cassy Workman, M.B., B.S., Janez Tomazic, M.D., Eva Jigel-Guedes, M.D., Sorin Rugini, M.D., Oleg Kozyrev, M.D., Juan Flores Cid, M.D., Phillip Hay, M.B., B.S., David Nolan, M.B., B.S., Sara Hughes, M.Sc., Arlene Hughes, Ph.D., Susanna Ryan, Ph.D., Nicholas Fitch, Ph.D., Daren Thorburn, Ph.D., and Alastair Benbow, M.B., B.S., for the PREDICT 1 Study Team

- First RCT to look at clinical utility of a pharmacogenetic test to prevent a specific drug toxicity
- Problem of false positive clinical diagnosis of abacavir hypersensitivity was overcome by using strategy of skin patch testing (100% specific) to define the phenotype of true immunologically mediated abacavir hypersensitivity
Prospective, randomized (1:1), **double-blind**, multicenter study with 6-week observation period (>90% of HSR cases)

**CONTROL**

ABC-naive subjects (n=1956)

No screening (n=976)

1:1 Randomization

ABC was the only required drug for this study; Remainder of regimen was investigator-selected

Patients start ABC (n=913); Samples tested for *HLA-B*<sup>5701</sup> at the end of the study

If HSR occurs, undergo patch test 6-8 weeks after event

**SCREENING**

Samples tested for *HLA-B*<sup>5701</sup> in real time (n=980)

Only *HLA-B*<sup>5701</sup>-subjects start ABC (n=859)

*HLA-B*<sup>5701</sup>+ subjects excluded from study (n=54)

Clinically Suspected and Immunologically Confirmed HSR in ITT evaluable population

- Clinically Suspected HSR
  - Control arm: 7.8% (66/847)
  - Prospective HLA-B*5701 screening arm: 3.4% (27/803)
  - OR 0.40 (0.25, 0.62) P < 0.0001

- Immunologically Confirmed HSR
  - Control arm: 2.7% (23/842)
  - Prospective HLA-B*5701 screening arm: 0.0% (0/802)
  - OR 0.03 (0, 0.18) P < 0.0001
Abacavir Exposed >6 weeks

Clinically suspected HSR 7.8%

Patch +ve HSR 2.7%

HLA-B*5701 +ve 'True' HSR (3.1%)

HLA-B*5701 +ve ABC Tolerant (2.6%)

100%

Abacavir Exposed >6 weeks


**HLA-B*5701 Carriage Frequency**

- **INDIA**: 5-20%
- **JAPAN**: 0%
- **CHINA**: 0%
  (NB 2.5% N.E. provinces)
- **UK**: ~8%
- **MIDDLE EAST**: 1-2%
  (NB 5-7% Ashkenazi Jews)
- **AUSTRALIA**: ~8%
- **US Caucasian**: ~8%
- **US Asian**: ~1%
- **US African-American**: ~2.5%
- **US Hispanic**: ~2%
- **W. EUROPE**: 5-7%
- **MEDITERRANEAN**: 1-2%
  (UK ~8%)
- **S. AMERICAN**: 5-7%
  (Caucasian)
- **Subsaharan AFRICA**: <1%
- **THAILAND**: 4-10%
  *THAILAND B*57 carriage:
  Urban Bangkok 3.6%
  Thai Dai Lue (NE Thai) ~11%
  Southern Thai Muslim 3%
- **MIDDLE EAST**: 1-2%
  (NB 5-7% Ashkenazi Jews)
- **CHINA**: 0%
  (NB 2.5% N.E. provinces)
- **JAPAN**: 0%
- **W. EUROPE**: 5-7%
- **AUSTRALIA**: ~8%

SHAPE Study Design

**CASES**

Black and White subjects with clinically-suspected ABC HSR (CS-HSR)

- ABC skin patch test & HLA-B*5701
  - Positive: White: 42, Black: 5
  - Negative: White: 85, Black: 63

**CONTROLS**

Black & White subjects enrolled in KLEAN, ALOHA, CNA30027, CNA30032

- Identify ABC-tolerant subjects who provided PGx consent and sample
  - White: 202, Black: 206

High Negative Predictive Value of HLA-B*5701 Generalised Across Race

**Sensitivity/Specificity of HLA-B*5701 and 95% CI**

- **OR: White**
  - CS-HSR: 1945 [110-34352]; SPT-pos: 19 [8-48]
- **OR: Black**
  - CS-HSR: 900 [30-21045]; SPT-pos: 17 [4-164]

**Control**

- **White**: 96%
- **Black**: 14%

**SPT-pos**

- **White**: 100%
- **Black**: 100%

**CS-HSR**

- **White**: 44%
- **Black**: 14%
**Preferred**

<table>
<thead>
<tr>
<th>NNRTI</th>
<th>EFV</th>
<th>ABC/3TC (for <em>HLA-B</em>(^{*}5701) negative patients) or TDF/FTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>FPV/r BID LPV/r BID ATV/r</td>
<td>ABC/3TC (for <em>HLA-B</em>(^{*}5701) negative patients) or TDF/FTC</td>
</tr>
</tbody>
</table>


IAS Guidelines:  *JAMA* 2008;300(5):555-70
Prerequisites for Successful Integration of HLA Pharmacogenetic Testing into Routine Clinical Care: Laboratory Aspects

- IP- freedom to operate
- development of simple, inexpensive, robust, yes/no laboratory tests and associated reagents (e.g., monoclonal antibodies)
- rapid and simple report and interpretation
- global distribution and commercialization of allele specific test
- quality assurance of allele specific test
- reimbursement of test

Phillips E, Mallal S. Curr Opin in Molecular Therapeutics 2009;3:231-42
## Available Technologies

<table>
<thead>
<tr>
<th>Available Technologies</th>
<th>Cost</th>
<th>Turn-around-time</th>
<th>Feasibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>High resolution HLA B typing</td>
<td>High</td>
<td>Long (2 weeks or more)</td>
<td>Not feasible unless specialty laboratory</td>
</tr>
<tr>
<td>PCR-based techniques</td>
<td>Moderate (+$100 USD)</td>
<td>Moderate (&lt;2 week)</td>
<td>Feasible for labs with molecular technologies</td>
</tr>
<tr>
<td>B17-monoclonal Flow Cytometry</td>
<td>Low</td>
<td>Low (mandated by need for fresh cells)</td>
<td>For labs doing CD4+/8</td>
</tr>
</tbody>
</table>

Pharmacogenetics and HLA: Predicting the Unpredictable?

- Type A reactions are predictable based on their pharmacological action therefore pharmacogenetic factors will only explain a proportion of the variability in drug response.
- Type B adverse drug reactions such as hypersensitivity reactions and severe skin syndromes are immunogenetically mediated.
- HLA associations are promising for prediction and hence prevention of these types of reactions but abacavir is currently the only drug where high level evidence exists and widespread screening has been implemented.
- Numerous clinical and laboratory hurdles must be overcome for successful integration of pharmacogenetic testing into a clinical setting.
Acknowledgments

Participants and clinical staff involved in the Western Australian HIV Cohort Study

Simon Mallal  James McCluskey
David Nolan Dianne Cheesman
Ian James Tess Lethborg
Mina John Tony Purcell
Annalise Martin Emma Hammond
Annette Patterson Mandvi Bharafway
Campbell Witt Richard Harrigan
Frank Christiansen Andri Rauch
Rom Kreuger Amalio Telenti
Susan Herrmann Hansjakob Furrer
Coral-Ann Almeida Julio Montaner

GSK and PREDICT-1 and SHAPE investigators and study teams

National Health and Medical Research Council of Australia

Participants and clinical staff involved in the Western Australian HIV Cohort Study

Simon Mallal  James McCluskey
David Nolan Dianne Cheesman
Ian James Tess Lethborg
Mina John Tony Purcell
Annalise Martin Emma Hammond
Annette Patterson Mandvi Bharafway
Campbell Witt Richard Harrigan
Frank Christiansen Andri Rauch
Rom Kreuger Amalio Telenti
Susan Herrmann Hansjakob Furrer
Coral-Ann Almeida Julio Montaner

GSK and PREDICT-1 and SHAPE investigators and study teams

National Health and Medical Research Council of Australia