Pharmacogenomic Testing for Predicting at Risk Patients - Genotyping and Flow Cytometry of HLA-B

Simon Mallal MBBS FRACP FRCPA
Professor and Director
Institute for Immunology and Infectious Diseases
Royal Perth Hospital and Murdoch University

AACC Annual Meeting, 22 July 2009, Chicago
Incorporation of a Pharmacogenetic Test into Clinical Practice

- High level evidence generalizable to diverse clinical settings
- Widespread availability of cost-effective and reliable laboratory tests
- Effective strategies to operationalize testing into routine clinical practice
Context of Results

Sensitivity of HLA-B*5701

- Mallal: 78%
- CNA30027: 57%
- CNA30032: 48%
- PREDICT-1: 48%
- SHAPE: 44%
- PHILLIPS: 25%
- PREDICT-1: 23%
- SHAPE: 42%
- IC-HSR: 5%

Mallal et al. Lancet 2002
Hughes et al. Pharmacogenomics 2004
Mallal et al. NEJM 2008
Saag et al. CID 2008
Phillips et al. IAS 2007 Abstract MOPEB001
**PHENOTYPIC UNCERTAINTY IN ABC HSR**

“Clinicians Are Hapless Phenotypers” Sir Sydney Brenner, Nobel Laureate

- Limits accurate assessment of the genotype-phenotype relationship
- Diagnostic imprecision versus Differential misclassification error

<table>
<thead>
<tr>
<th>Mallal <em>et al</em>, 2002</th>
<th>Hetherington <em>et al</em>, 2002*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HLA-B*5701</strong></td>
<td><strong>HLA-B57</strong></td>
</tr>
<tr>
<td><strong>HSR</strong></td>
<td><strong>No HSR</strong></td>
</tr>
<tr>
<td><strong>Pos</strong></td>
<td><strong>Neg</strong></td>
</tr>
<tr>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>177</td>
</tr>
<tr>
<td><strong>Sens 78%</strong></td>
<td><strong>Spec 97%</strong></td>
</tr>
<tr>
<td><strong>Pos PV</strong></td>
<td><strong>Neg PV</strong></td>
</tr>
<tr>
<td>74%</td>
<td>98%</td>
</tr>
</tbody>
</table>

| **Pos**                | **Neg**                     |
| 36                     | 29                           |
| 8                      | 649                          |

| **Sens 55%**           | **Spec 99%**                |
| **Pos PV**             | **Neg PV**                  |
| 82%                    | 96%                         |

- Clinical diagnosis sensitive but not specific
- Required adjunctive test that was specific (sensitivity was less critical)


**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><strong>HSR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1*</td>
<td></td>
</tr>
<tr>
<td><strong>No HSR</strong></td>
<td>4</td>
<td>180</td>
</tr>
</tbody>
</table>

Sens 93.8%  
Spec 97.8%

### Reclassified first 200 patients (*not available*)

<table>
<thead>
<tr>
<th></th>
<th>Pos PV</th>
<th>Neg PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>78.9%</td>
<td>99.4%</td>
<td></td>
</tr>
</tbody>
</table>
Abacavir Exposed >6 weeks
100%

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%)_
Screening Implications

**Black**
- n = 100
- HLA-B*5701 test
  - 2 Positive
    - Do not treat with ABC
  - 98 Negative
    - Appropriate to treat with ABC

**White**
- n = 100
- HLA-B*5701 test
  - 6 Positive
    - Do not treat with ABC
  - 94 Negative
    - Appropriate to treat with ABC

Test 100 Black patients:
- Treat 98 patients at low risk for ABC HSR
- Prevent 1 ABC HSR event
- Exclude ABC in 1 patient

Test 100 White patients:
- Treat 94 patients at low risk for ABC HSR
- Prevent 4 ABC HSR events
- Exclude ABC in 2 patients

Example shown is based upon PPV derived from PREDICT-1 and SHAPE data.
Incorporation of a Pharmacogenetic Test into Clinical Practice

- High level evidence generalizable to diverse clinical settings
- Widespread availability of cost-effective and reliable laboratory tests
- Effective strategies to operationalize testing into routine clinical practice
Specificity of HLA-B*5701 contributed by the 97 and 116 residues

<table>
<thead>
<tr>
<th>Polymorphic positions</th>
<th>4 9 11 12 24 32 41 45 46 52 62 63 65 66 67 69 70 71 74 76 77 80 81 82 83 94 95 96 97 99 103 108 113 114 116 131 145 152 156 158 159 163 167 171 177 178</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consensus</td>
<td>SYAMAQAEIERNQIYANADERSNLRGLTYVRHNLSRVLALWYET</td>
</tr>
<tr>
<td>HLA-B alleles in patients with Hsp70 Hom M493C in our cohort.* Saper et al 1991 JMB</td>
<td></td>
</tr>
</tbody>
</table>
Intracellular metabolism of abacavir

Oxidation of abacavir by ADH isoforms

May bind amine bearing residues in proteins via formation of a Schiff’s base or by cyclophilic attack

Adapted from Walsh et al., Chem Biol Interact 2002; 142(1-2): 135-154
**Abacavir bound peptides may mimic HLA-B57 epitopes**

<table>
<thead>
<tr>
<th>Start</th>
<th>End</th>
<th>HLA</th>
<th>Protein</th>
<th>Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>23</td>
<td>B*5701</td>
<td>p24</td>
<td>W</td>
</tr>
<tr>
<td>30</td>
<td>38</td>
<td>B*5701</td>
<td>Vpr</td>
<td>W</td>
</tr>
<tr>
<td>109</td>
<td>117</td>
<td>B*5701</td>
<td>p24</td>
<td>W</td>
</tr>
<tr>
<td>176</td>
<td>184</td>
<td>B*5701</td>
<td>p24</td>
<td>W</td>
</tr>
<tr>
<td>243</td>
<td>252</td>
<td>B*5701</td>
<td>RT</td>
<td>W</td>
</tr>
<tr>
<td>244</td>
<td>252</td>
<td>B*5701</td>
<td>RT</td>
<td>W</td>
</tr>
<tr>
<td>374</td>
<td>388</td>
<td>B*5701</td>
<td>RT</td>
<td>W</td>
</tr>
<tr>
<td>375</td>
<td>383</td>
<td>B*5701</td>
<td>RT</td>
<td>W</td>
</tr>
<tr>
<td>108</td>
<td>118</td>
<td>B*5701</td>
<td>p24</td>
<td>F</td>
</tr>
<tr>
<td>116</td>
<td>125</td>
<td>B*5701</td>
<td>Nef</td>
<td>Q</td>
</tr>
<tr>
<td>31</td>
<td>39</td>
<td>B*5701</td>
<td>Vif</td>
<td>F</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>B*5701</td>
<td>p24</td>
<td>F</td>
</tr>
<tr>
<td>14</td>
<td>23</td>
<td>B*5701</td>
<td>Rev</td>
<td>F</td>
</tr>
<tr>
<td>120</td>
<td>128</td>
<td>B*5701</td>
<td>Nef</td>
<td>T</td>
</tr>
</tbody>
</table>

**Non-polar properties of Abacavir and peptides**

<table>
<thead>
<tr>
<th>Non-polar properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>165.19</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>204.23</td>
</tr>
</tbody>
</table>
T cells from abacavir sensitive patients with HIV-1 are expanded in vitro in response to abacavir stimulation.

Day 1

PBMC + abacavir

Day 13

Restimulate responding T cells with and without abacavir loaded APC

Intracellular cytokine staining of T cells

Patient 1

- abacavir+

Patient 2

**Abacavir responses are HLA-B*5701 restricted**

<table>
<thead>
<tr>
<th>B*5701</th>
<th>B*5702</th>
<th>B*5801</th>
</tr>
</thead>
<tbody>
<tr>
<td>114Asp-Asn</td>
<td>116Ser-Tyr</td>
<td>45Met-Tyr</td>
</tr>
<tr>
<td>156Leu-Arg</td>
<td></td>
<td>46Ala-Glu</td>
</tr>
<tr>
<td></td>
<td>97Val-Arg</td>
<td>103Val-Leu</td>
</tr>
</tbody>
</table>

Residue 116 controls fine specificity of abacavir responses

B*5701  B*5702  B*5703  B*5701-114N  B*5701-116Y

114Asp → Asn  114Asp → Asn  114Asp → Asn  116Ser → Tyr
116Ser → Tyr  116Ser → Tyr
156Leu → Arg

IFNγ

28.4%  0.77%  1.34%  10.7%  0.59%

CD8

(1) Abacavir

Cytosol

APC

(2) Metabolism

Reactive metabolite

(3) Proteosome

(4) Haptenated peptide

(5) ER

HLSA-B*5701

Class I MHC

(6) Golgi

(7) Surface of APC

(8) Abacavir specific CD8+ T-cell

(9) Pro-inflammatory cytokines

(10) Hypersensitivity symptoms

Targeted Antigen Recognition is Likely to be a Critical Factor in Drug Hypersensitivity...

What the host immune system ‘sees’

"Bummer of a birthmark, Hal"
**HLA-B*5701 Carriage Frequency**

- **US**
  - Caucasian: ~8%
  - US Asian: ~1%
  - African-American: ~2.5%
  - Hispanic: ~2%
  - American Indian: ~2%

- **S. AMERICANS**
  - Caucasian: 5-7%
  - Sub-Saharan African: <1%

- **MIDDLE EAST**
  - 1-2%
  - (NB 5-7% Ashkenazi Jews)

- **UK**
  - ~8%

- **MEDITERRANEAN**
  - 1-2%

- **W. EUROPE**
  - 5-7%

- **INDIA**
  - 5-20%
  - (NB 2.5% N.E. provinces)

- **CHINA**
  - 0%

- **AUSTRALIA**
  - ~8%

- **JAPAN**
  - 0%

- **THAILAND**
  - 4-10%*

- **AFRICA**
  - 5-7%

- **US**
  - Asian: 25%

- **THAILAND B*57 carriage:**
  - Urban Bangkok 3.6%
  - Thai Dai Lue (NE Thai) ~11%
  - Southern Thai Muslim 3%

DNA Based High Resolution HLA-B*5701 Typing is Needed to Accurately Predict the Risk of HSR

Resolution of HLA-B*57 subtypes

B17 (serology)  \[\rightarrow\] B58  \[\rightarrow\] HLA-B58 = most common HLA-B allele in U.S. Asians

B57  \[\rightarrow\] HLA-B*5703 = allele specific to African populations

DNA based  \[\rightarrow\] B*5701  \[\rightarrow\] B*5702  \[\rightarrow\] B*5703
## 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 (&lt;$100 USD)</td>
<td>Moderate (&lt;2 week)</td>
<td>Feasible for labs with molecular technologies</td>
</tr>
<tr>
<td>B17-monoconal 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>

One hundred nanogram of genomic DNA was added, and the reaction mix was made up to a total volume of 25 µl. Touch-down amplification-cycling conditions on the MJ DNA Engine included at one cycle at 96°C for 1 min; 4 cycles of 96°C for 25 s, 70°C for 45 s and 72°C for 45 s; 24 cycles of 96°C for 25 s, 65°C for 50 s 72°C for 45 s; 8 cycles of 96°C for 25 s, 55°C for 1 min, 72°C for 1.20 min

The PCR products were electrophoresed on a 2% ultrapure agarose gel run alongside a 1 kb lambda size standard ladder for approximately 30 min at 150 V.

Amplification profiles discriminate HLA-B*5701 and HLA-B57 subtypes. Amplification was done using the True SNP SSP multiplex assay on DNA samples from individuals heterozygous for HLA-B57 subtypes and B-cell lines homozygous for HLA-B alleles.

SSP-PCR Methodology


Nucleotide sequence alignment of HLA-B alleles depicting the SSP primer positions.

PCR product one: HLA-B*5701, B*5514 (infrequent in WA), B*5708, B*5706^.

PCR product two: B57 subtypes –HLA B*5702, -B*5703, -B*5704, -B*5707, -B*5708, -B*5709 B*5709.

non-B57 subtypes- HLA B* 5514.

B5705 will not be amplified at all and B5706^ will amply product 1, but not 2.
Result Format

Matrix assessment of sensitivity and precision over varying conditions

- 1 cycle 96°C Denaturation
- 4 cycles 96°C Denaturation
- 70°C Annealing
- 72°C Extension
- 24 cycles 96°C Denaturation
- 65°C Annealing
- 72°C Extension
- 10 cycles 96°C Extension
- 55°C Annealing
- 72°C Extension
- Hold 15°C

- 2.5mM MgCl₂
- Varied concentration of primers 1 to 4 (HLA-B*57 and HLA-B*5701)
- Constant concentration for HGH primers (5pmol/µL)
- 2.5% agarose, 200V, 45 mins

Almeida and Mamotte, 2006
Matrix assessment of sensitivity and precision over varying conditions

**1 cycle**
- 96°C Denaturation
- 60-77°C Annealing
- 72°C Extension
- Hold 15°C

**4 cycles**
- 96°C Denaturation
- 61.5-56.5°C, 46.5°C Annealing
- 72°C Extension
- 72°C Extension

**24 cycles**
- 96°C Denaturation
- 64.8-59.8°C, 49.7°C Annealing
- 72°C Extension
- 72°C Extension

**10 cycles**
- 96°C Extension
- 72°C Extension

**Hold**
- 15°C

2.5% agarose, 200V, 45 mins

**Almeida and Mamotte, 2006**
<table>
<thead>
<tr>
<th>Annealing Temperature</th>
<th>Sample</th>
<th>Product</th>
<th>1.5mM</th>
<th>2.0mM</th>
<th>2.5mM</th>
<th>3.0mM</th>
<th>3.5mM</th>
<th>4.0mM</th>
<th>4.5mM</th>
<th>5.0mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>55.0°C</td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*57</td>
<td>HGH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>56.5°C</td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*57</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>59.8°C</td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*57</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>62.2°C</td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*57</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>65.1°C</td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*57</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>67.5°C</td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*57</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>70.7°C</td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*57</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>72.0°C</td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*57</td>
<td>HGH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701</td>
<td>HGH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Product +/-*  
**False positive**  
**False negative**  
**Assay failed**  

*Almeida and Mamotte, 2006*
**HLA-B*5701 Typing: Evaluation of an Allele-Specific PCR Melting Assay**

1a

- + + + + + + +
- + + + + + + +
- + + + + + + +

Primer 1, 2, 3, 5, 6

+ *HLA-B*5701 positive samples;
- *HLA-B*5701 negative samples

all amplicons plus negative controls

1b

[Graphs showing melting curves for different primer combinations and samples]
Rapid Screens for *HLA-B*\(^*57\) Identification

**B57/58 Flow cytometry**

- **Overlay of B17-IgG-FITC**
  - B57 negative
  - B5701 positive

**B*5701 SSP**

- B*46
- B*47
- B*60
- B*0702
- B*44
- B*53
- B*1302
- B*0801
- B*5701
- B*5704
- B*5703
- B*5703
- B*5702
- B*5702

---

*Martin A, et al.  
**Martin A, Nolan D, Mallal S.  
External quality assessment of HLA-B*5701 reporting: an international multicentre survey

Emma Hammond1*, Coral-Ann Almeida1, Cyril Mamotte2, David Nolan1, Elizabeth Phillips1, Tineke Asma Schollaardt3, M John GilP, Jonathan B Angel4, Doris Neurath4, Jianping Li4, Tony Giulivi4, Cathy McIntyre5, Galina Koulitchtski6, Betty Wong6, Marciano Reis6, Anita Rachiis6, David E Cole6, Choo Beng Chew7, Stefan Neifer8, Richard Lalonde9, Michel Roger10, Annie Jeanneau10 and Simon Malla11

1Centre for Clinical Immunology and Biomedical Statistics, Royal Perth Hospital and Murdoch University, Western Australia, Australia
2School of Biomedical Sciences, Curtin University of Technology, Western Australia, Australia
3University of Calgary, Calgary, AL, Canada
4Ottawa Hospital, Ottawa, ON, Canada
5Royal Victoria Hospital, Montreal, QC, Canada
6Sunnybrook and Women's College Health Sciences Centre, Toronto, ON, Canada
7Westmead Hospital, Westmead, New South Wales, Australia
8Centre for Microbiology and Infectious Epidemiology, Berlin, Germany
9Montreal Chest Hospital, Montreal, Quebec, Canada
10Laboratoire d'immunogénétique, Centre de Recherche du Centre Hospitalier de l'université de Montréal, Montréal, QC, Canada

Antiviral Therapy 2007; 12:1027-1032

***Ongoing quality assurance program mediated by ASEATTA

MDiviney@arcbs.redcross.org.au
Results: Two Groups of HLA-B*5701 Positives

PREDICT-1 (n=23)
SHAPE (n=47)
MNS (n=25)

Abacavir HSR patch positive
N=95 (all HLA*5701 positive)

1% abacavir
10% abacavir

HLA-B*5701 Positive Abacavir Tolerant

PREDICT-1 (n=19)
SHAPE (n=10)
MNS (n=14)

HLA-B*5701 positive abacavir tolerant
N=43

Nolan D et al, CROI 2008 Poster 982
Positive Predictive Value of HLA-B*5701

- HLA-B*5701 necessary but not sufficient for development of ABC HSR
- No complete surrogates for HLA-B*5701 within the MHC (decrease negative predictive value without increasing positive predictive value)

Nolan et al CROI 2008 Poster 982
Incorporation of a Pharmacogenetic Test into Clinical Practice

- High level evidence generalizable to diverse clinical settings
- Widespread availability of cost-effective and reliable laboratory tests
- Effective strategies to operationalize testing into routine clinical practice
Routine Pharmacogenetic Screening: Steps for Success

- Early and routine initiation of test
- Correct blood sample to correct lab
- Maintenance of sample and data integrity
- Robust assay
- Laboratory QAP
- Rapid, simple report and interpretation
- Education of HCWs and patients
- Information transmitted to, retained and acted on by
  - Clinician
  - Pharmacy
  - Patient
Translating Research into Clinical Practice: the ABacavir Example

**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 and Mallal, *Personalised Medicine* 2009, 6(4), 393-408
<table>
<thead>
<tr>
<th>Prerequisites</th>
<th>Drug/HLA association</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ABC</strong></td>
</tr>
<tr>
<td><strong>Test</strong></td>
<td></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* (i.e., 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 (e.g., 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 (e.g., 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>
Prerequisites for Successful Integration of HLA Pharmacogenetic Testing into Routine Clinical Care:
Attributes of Test, Drug and Environment

- Relatively prevalent HLA allele
- HLA allele prevalent in large, non-disenfranchised population
- Alternative drug(s) that do not need pharmacogenetic testing absent or have negative attributes
- Toxicity is readily and accurately phenotyped or an adjunctive diagnostic test such as skin patch testing can improve phenotypic precision
- Toxicity is severe and persistent (i.e., not isolated mild rash)
- Drug otherwise has favorable attributes
- Champions available, willing and able to drive pharmacogenetic test development and implementation

  E.g. Clinical academics, industry (if drug not off patent), professional bodies, regulatory agencies, guideline committees, patient advocacy groups, laboratory providers and media)
Prerequisites for Successful Integration of HLA Pharmacogenetic Testing into Routine Clinical Care: Attributes of Test, Drug and Environment

- Relatively prevalent HLA allele*
- HLA allele prevalent in large, non-disenfranchised population*
- Alternative drug(s) that do not need pharmacogenetic testing absent or have negative attributes*
- Toxicity is readily and accurately phenotyped* or an adjunctive diagnostic test such as skin patch testing can improve phenotypic precision
- Toxicity is severe and persistent* (ie not isolated mild rash)
- Drug otherwise has favorable attributes*
- Champions available, willing and able to drive pharmacogenetic test development and implementation
  
  E.g. Clinical academics, industry (if drug not off patent*), professional bodies, regulatory agencies, guideline committees, patient advocacy groups, laboratory providers and media)

*Non-modifiable
Prerequisites for Successful Integration of HLA Pharmacogenetic Testing into Routine Clinical Care: Generation of sufficient level of evidence

- case control studies
- population based cohort studies
- open studies of screening
- supportive basic science
- blinded randomized controlled trial
  - (level Ia evidence)
- evidence across ethnic groups
- cost-effectiveness data
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**
Prerequisites for Successful Integration of HLA Pharmacogenetic Testing into Routine Clinical Care: Clinical Aspects

- education of clinicians, nurses, pharmacists, phlebotomists, patients etc
- systems in clinic to ensure correct blood sample to correct lab
- systems to ensure test results and correct interpretation is rapidly transmitted to, retained by, and
- acted on by the healthcare team and patient

Phillips E, Mallal S, Current Opinion in Molecular Therapeutics 2009;11:231-42
## Acknowledgments

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elizabeth Phillips</td>
<td>Nebojsa Jojic</td>
</tr>
<tr>
<td>David Nolan</td>
<td>Tomer Hertz</td>
</tr>
<tr>
<td>Ian James</td>
<td>James McCluskey</td>
</tr>
<tr>
<td>Mina John</td>
<td>Tony Purcell</td>
</tr>
<tr>
<td>Annalise Martin</td>
<td>Emma Hammond</td>
</tr>
<tr>
<td>Mandvi Bharafway</td>
<td>Tess Lethborg</td>
</tr>
<tr>
<td>Campbell Witt</td>
<td>Richard Harrigan</td>
</tr>
<tr>
<td>Frank Christiansen</td>
<td>Andri Rauch</td>
</tr>
<tr>
<td>Amalio Telenti</td>
<td>Dianne Cheesman</td>
</tr>
<tr>
<td>Susan Herrmann</td>
<td>Hansjakob Furrer</td>
</tr>
<tr>
<td>Coral-Ann Almeida</td>
<td>Silvana Gaudieri</td>
</tr>
</tbody>
</table>

GSK and PREDICT-1 and SHAPE investigators and study teams

Bill and Melinda Gates Foundation
National Institutes of Health
National Health and Medical Research Council of Australia