CYP450 2C9 and VKORC1 for Enhancing Patient Outcomes with Warfarin Therapy
Warfarin Indications

- Atrial Fibrillation
  - 2.2 Million afflicted
- Venous Thrombosis - Pulmonary Embolism
  - Venous thrombosis requiring hospitalization
  - 600,000 hospitalizations / year for DVT
- Heart valve replacement
  - 95,000 valve procedures
- Acute Myocardial Infarction
  - Stroke or systemic embolization after MI
  - 865,000 new & recurrent attacks / year

3. Heart Disease and Stroke Statistics-- 2006 Update, American Heart Association, P. 36
4. Heart Disease and Stroke Statistics-- 2006 Update, American Heart Association, P. 2
Warfarin – the Pros

- Warfarin prevents 20 strokes for every bleeding episode that it causes\(^1\)

Warfarin – the Cons

- High number of reports for¹
  - Drug interactions
  - Medication errors
  - Serious bleeding
  - Hospital admissions

¹ Hamish Cameron, MD, VP of Exanta®, PPT Presentation to FDA = http://www.fda.gov/ohrms/dockets/ac/04/slides/2004-4069S1_01_A-Astra-Zeneca-Introduction.pdf
Spiraling Costs of Therapy

In-Patients

- $8000 per quality-adjusted life-year saved.¹
- $370,000 / quality-adjusted life-year saved¹ (65 yr olds)

Out-Patients

Just using the drug alone costs about $800 / year.²


Challenges in Warfarin Treatment

Numerous factors impact warfarin dosage

- Interactions with other drugs
- Patient age (older patients require lower dosage)
- Vitamin K dietary intake
- Individual patient variations

*Inhibition of Warfarin and 2C9: nafcillin, rifampin, griseofulvin, cholestyramine, barbiturates, carbamazepine, chlor Diazepoxide, sucralfate, dicloxacillin, phenylbutazone, sulfipyrazone, amiodarone, flucanozole, isoniazid, and ticlopidine. (Wells, Philip, et.al., Annals of Internal Medicine. 121(9) 676-682.)

Effective dosing for warfarin patients is a challenge because:

- Warfarin has a very complex dose-response relationship
- Warfarin has a very narrow therapeutic index
  - Most indications recommend an INR between 2.0 – 3.0
  - Dosage outside therapeutic levels, results in increased risk of clotting or bleeding*

Challenges in Warfarin Treatment

- Bleeding occurs in 6 to 39 % of recipients annually.¹
- Overdosing increases the risk of bleeding, including intracranial hemorrhage.²
- More than 7% of out-patients suffer a major hemorrhage¹
- Underdosing is associated with increased risk of thromboembolic complications.²

2. Del Negro, Albert A. Medscape Web MD. Warfarin has Role In Real World Clinical Practice for Prevention of Stroke for Patients with AF.
Challenges in Warfarin Treatment

- Prothrombin Time/Internationalized Normal Ratio (INR) monitored frequently until a dose sufficient to keep the INR within the desired range is achieved.
  - 1/3 of INR values > target therapeutic range in first month

- Once stabilized, the INR is monitored every 4-6 weeks

Vitamin K & Warfarin

- Warfarin blocks the formation of vitamin K-dependent clotting factors in the liver.

- Vitamin K is needed to make clotting factors that help the blood to clot and prevent bleeding.

Vitamin K = Vitamin K clotting factors = Warfarin Response

Vitamin K = Vitamin K clotting factors = Warfarin Response
Mark Rieder – “The management of warfarin therapy is complicated by a wide variation among patients in drug response. Variants in the gene encoding vitamin K epoxide reductase complex 1 (VKORC1) may affect the response to warfarin.”

Brian Gage – “Pharmacogenetic dosing (2C9) has the potential to decrease adverse events in patients starting warfarin.”

- Genetic analysis of a patient’s CYP450 2C9 and/or VKORC1 can be used to adjust warfarin dosage (1,2)

FDA’s Clinical Pharmacology Subcommittee of the Advisory Committee on Pharmaceutical Science (ACPS) recommended re-labeling for warfarin which will include testing of patients for genetic variants prior to initiating dosage.
Why Re-label Warfarin?

**DOSING SCHEDULES**

- **Initial Dose:** 35 mg/week
  - Age
  - Gender
  - BSA
  - Concomitant Drugs
  - Co-morbidities

**Stable Maintenance Dose**
- 29 mg/wk
- 28 mg/wk
- 24 mg/wk
- 18 mg/wk
- 6 mg/wk

**Repeat INR: Adjust Dose**
- Increase
- Decrease

- 30-35%
- 20-25%

**INR**

30 Days
Relevance of CYP 450 2C9 & Warfarin

- CYP2C9 - Isoenzyme for metabolizing warfarin
  - Many studies have used 2C9 to predict patient response

- CYP2C9 will allow:
  - Dose recommendations to reduce risk of adverse drug rxns.
  - Screening of high-risk patients requiring lower initial doses.
Predicting the Warfarin Stable Dose by adding CYP2C9 genotype

What about VKORC1 Genotype?

<Modified from Caldwell M., CPSC Advisory Committee Meeting, November 14, 2005>
http://www.fda.gov/ohrms/dockets/ac/05/slides/8>
Effect of CYP2C9 Genotype on Warfarin Maintenance Dose

Median stabilization follow-up visits = 23 times
Median time span of follow-up visits = 543 days

Daily maintenance Dose (mg)

*1*1 [69%]  *1*2 [15%]  *2*2 [2%]  *1*3 [10%]  *2*3 [1.6%]  *3*3 [2.7%]

2C9 Genotype Group Percentages
N = 185

< Adapted from Higashi MK et al, JAMA 2002; 287:1690>
Clinical Relevance of VKORC1

- Vitamin K Epoxide Reductase Complex 1 is a protein that helps control blood clotting.

- Variants effect warfarin response

- “VKORC1 Haplotypes can be used to stratify patients into low, intermediate, and high dose warfarin groups and may explain differences in dose requirements among patients of different ancestries.”

Detecting variants of 2C9 plus VKORC1 will allow physicians to better adjust dosing:

- VKORC1 accounts for 29-30% of dose variation\(^1\)
- 2C9 accounts for 12% of dose variation\(^1\)

Warfarin Dosing – Using 2C9 & VKORC1

CYP2C9 and VKORC1 on Warfarin Stable Dose

<Modified from Caldwell M., CPSC Advisory Committee Meeting, November 14, 2005, http://www.fda.gov/ohrms/dockets/ac/05/slides/8>
Effect of CYP2C9 (*1, 2, 3) and VKORC1 (-1639G>A) on Warfarin Dose

Distribution of warfarin dose by CYP2C9 and VKORC1 genotype

\[ N=297 \quad [56\%] \]

\begin{align*}
*1*1 & : 22\% \\
*1*2 & : 3\% \\
*2*2 & : 14\% \\
*1*3 & : 5\% \\
*2*3/*3*3 & : 19\%
\end{align*}

< Adapted from Sconce et al, Blood, October 2005>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>VKORC1 Variants:</td>
<td>861, 3673, 5808, 6009, 6484, 6853, 7566, 8773, 9041</td>
</tr>
<tr>
<td>Sample:</td>
<td>Whole Blood/50 ng per reaction</td>
</tr>
<tr>
<td>Sample Preparation:</td>
<td>Single Multiplex 13-plex PCR For All 2C9 and VKORC1 Variants</td>
</tr>
<tr>
<td>Method of SNP Δ:</td>
<td>Detection Primer Extension Polymerase mediated genotyping</td>
</tr>
<tr>
<td>Method of Hybridization:</td>
<td>BioFilmChip™ Zip Code / Anti-Zip Code</td>
</tr>
</tbody>
</table>
Significance of 2C9 Variants

➢ **Commonly Identified Variants**

- **CYP2C9 *2, *3**

➢ **Other Variants Needed**

- **CYP2C9 *4, *5, *6**
  - CYP2C9 *4 - exclusively identified in Japanese population
  - CYP2C9 *5 and CYP2C9*6 - African Americans

- **CYP2C9 *11**
  - The CYP2C9 *11 - occurs in 1% of Caucasian and African-American populations

  CYP2C9*11 must be included in routine test panels for genotyping of oral anticoagulant patients.

  *1/ *11 (n=127) exhibited a 33% reduction in warfarin maintenance dose

➢ **Utilizing an expanded panel of 2C9 variants may identify more at risk patients which may prevent potential bleeding events**

2. [http://www.healthanddna.com/professional/2C9testmenu.htm](http://www.healthanddna.com/professional/2C9testmenu.htm)
Significance of VKORC1 Variants

- **Commonly Identified Variants**
  - $3673 = (-1639G>A)$

- **Other Variants Needed**
  - SNPs identified at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041 of the VKORC1 (Bold type indicate INFINITI variants detected)
    - “VKORC1 haplotypes can be used to stratify patients into low, intermediate, and high dose warfarin groups and may explain differences in dose requirements among patients of different ancestries.”
  - 8773 is present in 21% of African Americans.

- **Utilizing an expanded panel of VKORC1 variants may identify more at risk patients which may prevent potential bleeding events**

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### VKORC1 Haplotypes

#### Table 1. VKORC1 Haplotype Frequency and Effect on Warfarin Dose among 186 European-American Patients.*

<table>
<thead>
<tr>
<th>Haplotype Identification Code</th>
<th>Haplotype Sequence</th>
<th>Frequency of Haplotype in Primary Patient Population</th>
<th>Mean Maintenance Dose among Homozygous Patients (95% CI) ‡</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>proportion</td>
<td>no. of persons</td>
<td>mg/day</td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>CCGATCTCTCTG</td>
<td>0.12</td>
<td>2.9 (2.2–3.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>H2</td>
<td>CCGAGCTCTCTG</td>
<td>0.24</td>
<td>3.0 (2.5–3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>H3</td>
<td>CCGGTCCCCG</td>
<td>0.01</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H4</td>
<td>CCGGTCCGCG</td>
<td>&lt;0.01</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H5</td>
<td>TCGAAGCTCTG</td>
<td>&lt;0.01</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H6</td>
<td>TCGGTCCGCG</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>H7</td>
<td>TCGGTCCGCA</td>
<td>0.35</td>
<td>6.0 (5.2–6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>H8</td>
<td>TAGGTCCGCA</td>
<td>0.08</td>
<td>4.8 (3.4–6.7)</td>
<td>0.76</td>
</tr>
<tr>
<td>H9</td>
<td>TACGTTCCGCG</td>
<td>0.21</td>
<td>5.5 (4.5–6.7)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* CI denotes confidence interval, and NA not analyzed.
† For each haplotype sequence, the single-nucleotide polymorphisms are listed in sequence, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041.
‡ Analyses were adjusted for age, sex, use or nonuse of amiodarone, use or nonuse of NSAID.

**Key Points:**
- M. Rieder, NEJM 2005
- 10 SNP’s necessary to identify 9 haplotypes
- Correlating haplotype to warfarin dose was only done with European-American patients

**Key Points:**

100% correlation between INFINITI SNP identification and bi-directional sequencing.

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**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>H1</th>
<th>H2.5</th>
<th>H3</th>
<th>H4</th>
<th>H6</th>
<th>H7</th>
<th>H7*</th>
<th>H8</th>
<th>H9</th>
<th>NEW</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infiniti System</strong></td>
<td>48</td>
<td>70</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>72</td>
<td>16</td>
<td>18</td>
<td>46</td>
<td>2</td>
<td>294</td>
</tr>
<tr>
<td><strong>Bi-Directional Sequencing</strong></td>
<td>48</td>
<td>70</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>72</td>
<td>16</td>
<td>18</td>
<td>46</td>
<td>2</td>
<td>294</td>
</tr>
</tbody>
</table>

**VKORC1 Haplotypes (4)**

<table>
<thead>
<tr>
<th>H1</th>
<th>C</th>
<th>A</th>
<th>T</th>
<th>C</th>
<th>T</th>
<th>C</th>
<th>C</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2.5</td>
<td>C</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>T</td>
<td>G</td>
</tr>
<tr>
<td>H3</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>H4</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>H6</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>H7</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>H7*</td>
<td>C</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>H8</td>
<td>A</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>H9</td>
<td>A</td>
<td>G</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

**2C9 Mutations**

<table>
<thead>
<tr>
<th>cDNA Nucleotide Change</th>
<th>CYP2C9*2</th>
<th>CYP2C9*3</th>
<th>CYP2C9*4</th>
</tr>
</thead>
<tbody>
<tr>
<td>430 C&gt;T</td>
<td></td>
<td>1075 A&gt;C</td>
<td>1076 T&gt;C</td>
</tr>
<tr>
<td>.</td>
<td>CYP2C9*5</td>
<td>CYP2C9*6</td>
<td>CYP2C9*11</td>
</tr>
<tr>
<td>1080 C&gt;G</td>
<td></td>
<td>818 delA</td>
<td>1003 C&gt;T</td>
</tr>
</tbody>
</table>

*Bold SNP are Variant from the reference Allele*

**Table 2**

**Table 3**

**Table 4**

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When haplotyping African-American patients, it is clear that the H3, H4, H6, and H7* haplotypes all show significant presence, while they are practically non-existent in Rieder’s study.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency of Haplotype in Population According to Rieder Data</th>
<th>Frequency of Haplotype in African-American Population According to AutoGenomics Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>12%</td>
<td>6.90%</td>
</tr>
<tr>
<td>H2</td>
<td>24%</td>
<td>8.09%</td>
</tr>
<tr>
<td>H3</td>
<td>1%</td>
<td>16.18%</td>
</tr>
<tr>
<td>H4</td>
<td>&lt;1%</td>
<td>6.94%</td>
</tr>
<tr>
<td>H5</td>
<td>&lt;1%</td>
<td>N/A</td>
</tr>
<tr>
<td>H6</td>
<td>N/A</td>
<td>12.72%</td>
</tr>
<tr>
<td>H7</td>
<td>35%</td>
<td>24.28%</td>
</tr>
<tr>
<td>H7*</td>
<td>N/A</td>
<td>17.34%</td>
</tr>
<tr>
<td>H8</td>
<td>8%</td>
<td>1.15%</td>
</tr>
<tr>
<td>H9</td>
<td>21%</td>
<td>6.90%</td>
</tr>
</tbody>
</table>
When haplotyping Asian patients, the H1 & H7 haplotypes are the only two of interest. This data shows that most Asians fall under the H1 haplotype, which is a significant increase in comparison to European-Americans (as studied by Rieder, et. al).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency of Haplotype in Population According to Rieder Data</th>
<th>Frequency of Haplotype in Asian Population According to AutoGenomics Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>12%</td>
<td>94.12%</td>
</tr>
<tr>
<td>H2</td>
<td>24%</td>
<td>N/A</td>
</tr>
<tr>
<td>H3</td>
<td>1%</td>
<td>N/A</td>
</tr>
<tr>
<td>H4</td>
<td>&lt;1%</td>
<td>N/A</td>
</tr>
<tr>
<td>H5</td>
<td>&lt;1%</td>
<td>N/A</td>
</tr>
<tr>
<td>H6</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>H7</td>
<td>35%</td>
<td>5.88%</td>
</tr>
<tr>
<td>H7*</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>H8</td>
<td>8%</td>
<td>N/A</td>
</tr>
<tr>
<td>H9</td>
<td>21%</td>
<td>N/A</td>
</tr>
</tbody>
</table>
## Haplotype Distribution in Caucasians

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Frequency of Haplotype in Population According to Rieder Data</th>
<th>Frequency of Haplotype in Caucasian Population According to AutoGenomics Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>12%</td>
<td>18.39%</td>
</tr>
<tr>
<td>H2</td>
<td>24%</td>
<td>25.69%</td>
</tr>
<tr>
<td>H3</td>
<td>1%</td>
<td>2.29%</td>
</tr>
<tr>
<td>H4</td>
<td>&lt;1%</td>
<td>N/A</td>
</tr>
<tr>
<td>H5</td>
<td>&lt;1%</td>
<td>N/A</td>
</tr>
<tr>
<td>H6</td>
<td>N/A</td>
<td>0.46%</td>
</tr>
<tr>
<td>H7</td>
<td>35%</td>
<td>23.85%</td>
</tr>
<tr>
<td>H7*</td>
<td>N/A</td>
<td>0.92%</td>
</tr>
<tr>
<td>H8</td>
<td>8%</td>
<td>9.87%</td>
</tr>
<tr>
<td>H9</td>
<td>21%</td>
<td>19.73%</td>
</tr>
</tbody>
</table>

The AutoGenomics Caucasian population data is very similar to that of Mark Rieder’s from the New England Journal of Medicine.
The AEI-Brookings Joint Center for Regulatory Studies estimates that formally integrating genetic testing into routine warfarin therapy:

- Could allow American warfarin users to avoid 85,000 serious bleeding events and 17,000 strokes annually, and the cost savings would be **1.1 billion** annually.¹

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Conclusion

- Detecting 2C9 variants in addition to *2, *3 is essential for a broader identification of high risk warfarin metabolizers in several ethnic groups.
    - Recent publication in 2006 suggests that CYP2C9*11 must be included in routine testing for warfarin sensitivity.

- Detecting VKORC1 variants in addition to 3673(D-1639G>A) is important for improved haplotype identification in a diverse ethnic population.
  - 381, 861, 5808, 6009, 6484, 6853, 7566, 8773, 9041
    - Recent publications in 2006 suggests that 381 distinguishes 2 major haplotypes for Asians and 8773 is significantly present in African-Americans.

- Comprehensive panel of genetic variants for 2C9 & VKORC1 allows for enhanced characterization of patients with diverse ethnic backgrounds
INFINITI 2C9-VKORC1 Panel

• **Plug N Play** automation
• **Load N Go** with results within 5 hours
• **Characterize** patients of diverse ethnicities
INFINITI™ 2C9-VKORC1 Panel

SPOT ON

AGILITY - Rapid Turnaround

CONFIDENCE - Reliable

EFFICIENCY - Load & Go