Faculty Disclosure Information Elements

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Title of Presentation Molecular Clocks

Name of Commercial Interest No relevant financial relationship exists
Unwinding the Clock in Cardio-Metabolic Disease

ATVB Meeting
Nashville
Clock fundamentals

• Master clock in the Supra Chiasmatic Nucleus
• Clocks in all peripheral tissues except the testis
• Diverse signals from the center can entrain the periphery which retains a capacity for autonomy
• Environmental cues adjust the clock to a 24 hour rhythm – light, temperature, food
• It’s an anticipatory system
• But why should you care…..?
Clocks are highly conserved in evolution
Clockworks are tightly regulated

Yang et al Sci Trans Med 5;212rv3, 2013
Redundancy is built in to a highly regulated System
siRNA KD defines Network Features of Mammalian Clock
Bmal – Luc Oscillation

Clockworks knit together Biological Networks across Tissues.

- Water channel controlled by hypothalamus Aquaporin 5.
- Immune response signaling through interferon family genes.
- Stress module under adipose heat shock genes Hsp110 and Dnajb1.
- Circadian rhythm centered on Arntl gene.
- Autonomic function changes controlled by hypothalamus Phox2a.
- Energy balance involves AgRP and NPY.

E. Schadt
## Clock-regulated drug targets

<table>
<thead>
<tr>
<th>Rank</th>
<th>Sales</th>
<th>Trade name</th>
<th>Drug</th>
<th>Indications</th>
<th>Circadian-gene targets</th>
<th>Organs in which targets oscillate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>$1.46 b</td>
<td>Nexium</td>
<td>esomeprazole</td>
<td>Gastritis, GERD, Esophagitis</td>
<td>Apa4a</td>
<td>liver</td>
</tr>
<tr>
<td>5</td>
<td>$1.28 b</td>
<td>Advair Diskus</td>
<td>fluticasone + salmeterol</td>
<td>Asthma, Chronic obstructive pulmonary dis...</td>
<td>Adb2</td>
<td>kidney, lung, skeletal muscle</td>
</tr>
<tr>
<td>11</td>
<td>$794 m</td>
<td>Rituxan</td>
<td>rituximab</td>
<td>Rheumatoid arthritis, Non-Hodgkin's lymph...</td>
<td>Fcgry2b, Ms4a1</td>
<td>kidney, skeletal muscle</td>
</tr>
<tr>
<td>20</td>
<td>$538 m</td>
<td>Diovan</td>
<td>valsartan + hydrochlorothiazide</td>
<td>Hypertension, Heart failure</td>
<td>Kenm1</td>
<td>liver</td>
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<tr>
<td>27</td>
<td>$431 m</td>
<td>Vyvanse</td>
<td>lisdexamfetamine</td>
<td>Attention deficit hyperactivity disorder</td>
<td>Adb1a</td>
<td>liver</td>
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<tr>
<td>32</td>
<td>$392 m</td>
<td>Tamiflu</td>
<td>oseltamivir</td>
<td>Influenza</td>
<td>Neu1, Neu2</td>
<td>liver, kidney, lung, cerebellum</td>
</tr>
<tr>
<td>33</td>
<td>$383 m</td>
<td>Ritalin</td>
<td>methylphenidate</td>
<td>Attention deficit hyperactivity disorder</td>
<td>Scl6a4</td>
<td>kidney, adrenal gland</td>
</tr>
<tr>
<td>37</td>
<td>$348 m</td>
<td>AndroGel</td>
<td>testosterone</td>
<td>Hypogonadism</td>
<td>Ar</td>
<td>brown fat, adrenal gland</td>
</tr>
<tr>
<td>38</td>
<td>$346 m</td>
<td>Lidoderm</td>
<td>lidocaine</td>
<td>Pain</td>
<td>Egfr</td>
<td>heart</td>
</tr>
<tr>
<td>44</td>
<td>$304 m</td>
<td>Seroquel XR</td>
<td>quetiapine</td>
<td>Bipolar disorder, Major depressive disor...</td>
<td>Htr2a, Dvd4, Htr2c, ...</td>
<td>liver, kidney, lung, brown fat, heart, a...</td>
</tr>
<tr>
<td>45</td>
<td>$289 m</td>
<td>Viagra</td>
<td>sildenafil</td>
<td>Erectile dysfunction</td>
<td>Pde5a, Pde9g</td>
<td>brown fat, adrenal gland</td>
</tr>
<tr>
<td>47</td>
<td>$281 m</td>
<td>Nicotin</td>
<td>nicin</td>
<td>Hyperlipidemia</td>
<td>Qprt</td>
<td>kidney</td>
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<tr>
<td>48</td>
<td>$279 m</td>
<td>Humalog</td>
<td>insulin lispro</td>
<td>Diabetes mellitus T2</td>
<td>Igf1r</td>
<td>kidney</td>
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<tr>
<td>49</td>
<td>$274 m</td>
<td>Alimta</td>
<td>pemetrexed</td>
<td>Mesothelioma, Non-small cell lung cancer</td>
<td>Tmys, Galt, Atic</td>
<td>liver, lung, aorta</td>
</tr>
<tr>
<td>54</td>
<td>$267 m</td>
<td>Combitvent</td>
<td>ipratropium bromide + salbutamol</td>
<td>Asthma, Chronic obstructive pulmonary dis...</td>
<td>Chrm2, Adb2, Adb1</td>
<td>kidney, lung, heart, skeletal muscle, br...</td>
</tr>
<tr>
<td>56</td>
<td>$262 m</td>
<td>ProAir HFA</td>
<td>salbutamol</td>
<td>Asthma, Chronic obstructive pulmonary dis...</td>
<td>Adb2, Adb1</td>
<td>kidney, lung, skeletal muscle</td>
</tr>
<tr>
<td>62</td>
<td>$240 m</td>
<td>Janumet</td>
<td>metformin + sitagliptin</td>
<td>Diabetes mellitus T2</td>
<td>Prkab1, Dpp4</td>
<td>kidney, heart, brainstem, hypothalamus</td>
</tr>
<tr>
<td>66</td>
<td>$236 m</td>
<td>Toprol XL</td>
<td>metoprolol</td>
<td>Hypertension, Heart failure</td>
<td>Adb2, Adb1</td>
<td>kidney, lung, skeletal muscle</td>
</tr>
<tr>
<td>71</td>
<td>$220 m</td>
<td>Vytovin</td>
<td>ezetimbe + simvastatin</td>
<td>Hyperlipidemia</td>
<td>Anpep, Sosp1, Hnger</td>
<td>liver, lung, brainstem</td>
</tr>
<tr>
<td>78</td>
<td>$209 m</td>
<td>Aciplex</td>
<td>rabeprazole</td>
<td>Gastritis, GERD, Esophagitis</td>
<td>Apa4a</td>
<td>liver</td>
</tr>
<tr>
<td>90</td>
<td>$189 m</td>
<td>Lunesta</td>
<td>eszopiclone</td>
<td>Insomnia</td>
<td>Topo, Gabra3</td>
<td>kidney, lung, adrenal gland</td>
</tr>
<tr>
<td>98</td>
<td>$173 m</td>
<td>Prilosec</td>
<td>omeprazole</td>
<td>Gastritis, GERD, Esophagitis</td>
<td>Apa4a</td>
<td>kidney, adrenal gland</td>
</tr>
<tr>
<td>99</td>
<td>$171 m</td>
<td>Focalin XR</td>
<td>dexamethasphenidate</td>
<td>Attention deficit hyperactivity disorder</td>
<td>Scl6a4</td>
<td>kidney, adrenal gland</td>
</tr>
</tbody>
</table>

### Table 1: Drugs from the top-100 best-selling drugs list that target circadian genes AND have half-life < 6h. For full table, see Dataset S1. Rank and sales are based on USA 2013 Q1 data from Drugs.com.
Time dependent hypotensive effect of low dose aspirin

[Image of bar charts showing the effect of aspirin administration time on blood pressure, with significance levels indicated.]

Hermeida group  Chronobiol Int 2013 Mar;30(1-2):260-79
Time dependent hypotensive effect of low dose aspirin in mice on HSD

Wang et al 2016
A circadian gene expression atlas in mammals: Implications for biology and medicine
Ray Zhang\textsuperscript{a,1}, Nicholas F. Lahens\textsuperscript{a,1}, Heather I. Ballance\textsuperscript{a}, Michael E. Hughes\textsuperscript{b,2}, and John B. Hogenesch\textsuperscript{a,2}

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Edited by Joseph S. Takahashi, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, and approved September 19, 2014 (received for review May 13, 2014)

Ptgs1 (COX-1)

UGUCAGAAGUUG--ACCGUCGAA

CCCATCCAGATCTTTGCTTGTGGCAGCTGTTTCT

mir22
How we were…

- Clear distinction between light and dark
- Clear thermal contrasts
- We ate, quickly, what we killed
How we are...

- Continuous access to food
- Living in controlled thermal environments
- Jet lag, shift work
- Night lights, cell phones, tablets…
Oscillator subsets in Aortic Tissue

Insulin secretion

Diabetes

Polyunsaturated fatty acid synthesis

Obesity

Attenuated feeding rhythm

Yang et al Sci Trans Med 5;212rv3, 2013
Peripheral Regulation of Central Behavior

Bmal1 deletion abolishes oscillations in microbial abundances

**Bacteroidetes**

**Firmicutes**

**B/F Ratio**

$p = 0.00749$

$p = 0.01069$

$p = 0.00749$

$p = 0.01718$

$p = 0.01069$
Gender dependent impact of clock disruption on bacterial abundance

Liang et al PNAS 12(33):10479-84, 2015
The temporal levels of Bmal1 in a macrophage counters inflammation in a time dependent manner.

Dawn macrophage (ZT0) - Less cytokines and chemokines, Less sepsis

Dusk macrophage (ZT12) - More cytokines and chemokines, More sepsis
BMAL1 in the myeloid lineage is responsible for the morning protection of LPS

Curtis et al PNAS 2015 Jun 9;112(23):7231-6

ZT0 = DAWN = Bmal1 high
ZT12 = DUSK = Bmal1 low
Disruption of positive limb function causes neuropathology

**BMAL1 KO:**
- Severe astroglisis
- Late microglial activation
- Synaptic degeneration
- Impaired neural network function

Musiek et al JCI 123, 5389-400, 2013
Functional connectivity deficits in *BMAL1 KO* mice

Resting-state functional connectivity optical intrinsic signal imaging (fcOIS)-Culver lab

Disordered clocks…

• Metabolic dysfunction
• Disrupted immunoregulation
• Neurodegeneration and accelerated aging

Based largely on deletion of the one non-redundant core clock gene, Bmal1. Conventional knockouts have a lifespan roughly 1/3 of normal
The loss of circadian rhythms in Bmal1 iKO mice.
Survival unaltered by postnatal depletion of Bmal1

Contrasting phenotypes in cKO and iKO mice.
Summary

• Despite similar depletion of clock genes and loss of behavioral and cardiovascular oscillatory phenotypes, survival is markedly different in mice in which Bmal-1 is deleted pre- or postnatally.

• While some aging phenotypes are retained, others are reversed in the iKOs.

• Many more genes dysregulated in the cKOs, suggesting many phenotypes attributed to the clock may reflect off target effects of Bmal-1 during development.
Diurnal Variability in the Human Condition

- Heart attack, stroke
- Depression, Suicide and Suicidal attempts
- Asthma
- Arthritis pains
- Memory retrieval, cognitive function
- Exercise preference
- Eating and drinking behavior
The Human Chronobiome

• Integration of omics and remote sensing approaches to determine signal:noise detection in humans “in the wild”.

• Determination of variance, integration of heterogeneous data and examination of age and sex as variables.

• Basal and evoked phenotypes in healthy individuals a basis for seeking divergence in time dependent expression of disease.
Guangrui, Xue and Carsten
Truth is the daughter of time and I feel no shame in being her midwife

Johannes Kepler