Dr Romas Geleziunas

Gilead Sciences Inc, USA
Dr Romas Geleziunas

Gilead Sciences Inc, USA

COMPETING INTEREST OF FINANCIAL VALUE > £1,000:

<table>
<thead>
<tr>
<th>Speaker Name</th>
<th>Statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roman Geleziunas</td>
<td>Romas Geleziunas is an employee of Gilead Sciences, Inc.</td>
</tr>
<tr>
<td>Date</td>
<td>April 2013</td>
</tr>
</tbody>
</table>
Towards a Cure

Romas Geleziunas, Ph.D.
Director, Clinical Virology
Gilead Sciences, Inc.

April 17, 2013
Potential Strategy to Eradicate Latently Infected Cells

1. Activate HIV expression in latently infected cells
   - De-repress chromatin: Inhibit HDACs
   - Activate transcription factors (NF-κB)
   - Activate HIV mRNA elongation (PTEF-b)

2. Eliminate cells actively replicating HIV
   - Viral cytopathic effects
   - Virus-specific immune responses
   - Virus-directed toxic agents

3. Infection by newly produced virus particles blocked by ARVs (Intensified)

*Richman et al., Science 323 (2009)*
Activating and Eliminating HIV Reservoirs

♦ Activating HIV expression in latently infected cells
  - HDAC inhibitors (Romidepsin)
  - PKC activators
  - BRD4 inhibitors
  - HTS (NCEs)

♦ Eliminating cells actively replicating HIV
  - Immune modulators (TLR7 Agonists)
  - HIV mAbs (ADCC, Phagocytosis), bispecifics, ADCs
  - Therapeutic vaccines

♦ Combinations
Activating HIV expression in latently infected cells: Romidepsin (RMD)
Histone Deacetylases (HDACs) and Latent HIV

- Family of zinc metalloenzymes
- Catalyze removal of acetyl groups from lysine
- Cellular substrates: Histones, Tubulin, Transcription factors
- HDAC inhibitors activate latent HIV

HDACs are Recruited to the HIV LTR

Histone acetylation status influences chromatin structure and gene expression

Transcription factors and PTEF-b?
## HDACis Selected for Testing in HIV+ Subjects on ART

<table>
<thead>
<tr>
<th>Structure</th>
<th>Regulatory Status</th>
<th>Indication</th>
<th>Dose</th>
<th>Clinical studies in HIV+ Subjects</th>
<th>Dose / Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat (SAHA, Merck)</td>
<td>FDA Approved</td>
<td>CTCL</td>
<td>400 mg QD PO</td>
<td>Underway (US &amp; Australia)</td>
<td>400mg (single &amp; 14 days)</td>
</tr>
<tr>
<td>Romidepsin (Celgene)</td>
<td>FDA Approved</td>
<td>CTCL</td>
<td>14 mg/m² IV Days 1, 8, 15 (28 day cycle)</td>
<td>Clinical protocol development (US-ACTG)</td>
<td>&lt; 14 mg/m² SAD</td>
</tr>
<tr>
<td>Panobinostat (Novartis)</td>
<td>Phase 3</td>
<td>MM/AML</td>
<td>20mg TIW PO</td>
<td>Underway (Denmark)</td>
<td>20mg TIW every other week / 8 weeks</td>
</tr>
</tbody>
</table>
In Vitro Activation of HIV by HDAC Inhibitors

RMD (900x) and Panobinostat (400x) are more potent than Vorinostat. Potency to activate HIV correlates with potency to inhibit HDACs 1,2,3,10,11.

<table>
<thead>
<tr>
<th>Drug</th>
<th>EC$_{50}$ (nM)</th>
<th>CC$_{50}$ (nM)</th>
<th>%max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romidepsin</td>
<td>4.49</td>
<td>107</td>
<td>98%</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>10.1</td>
<td>&gt; 2,500</td>
<td>108%</td>
</tr>
<tr>
<td>Givinostat</td>
<td>95.8</td>
<td>24,000</td>
<td>79%</td>
</tr>
<tr>
<td>SB939</td>
<td>212</td>
<td>&gt; 50,000</td>
<td>104%</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>3,950</td>
<td>&gt; 25,000</td>
<td>100%</td>
</tr>
<tr>
<td>Mocetinostat</td>
<td>13,600</td>
<td>10,100</td>
<td>75%</td>
</tr>
</tbody>
</table>
**Ex vivo HIV Activation Assay with CD4+ T-cells from HIV+ Subjects on cART**

1. **Leukapheresis**
   - HIV+ subjects (cART)
   - ~$10^9$ PBMCs

2. **Cell isolation**
   - with Magnetic Beads

3. **Memory or resting CD4+ T-cells**
   - 2 – 5 million cells/well
   - + compounds & EFV/EVG

4. **Supernatants containing virions**

5. **Cell lysates**

6. **Roche COBAS AmpliPrep/TaqMan**
   - isolates and measures HIV vRNA

7. **QIAsymphony**
   - total RNA extraction w/ DNase I digestion
Ex Vivo HIV Activation by RMD and SAHA

Memory CD4+ T-cells (Leucopak): Pulse Treatment

- **Cell-associated vRNA**
  - Pulse: 4h, 24h
  - Analysis: 48h
  - Relative to clinical exposures: 5 – 40 nM RMD (5 – 40%), SAHA 0.5 – 1 µM (100 – 200%)

- **Extracellular vRNA**
  - Pulse: 4h, 24h
  - Analysis: 6 days

- HIV activation (fold above ctrl)
  - RMD 40nM
  - SAHA 1µM

- RMD 5nM, RMD 40nM
- SAHA 0.5µM, SAHA 1µM
Persistent HIV Expression Following RMD Treatment

CD4+ T-cells (Leucopaks): Cell-associated vRNA

Memory CD4+ T-cells Pulse Treatment

Resting CD4+ T-cells Pulse Treatment
HIV Activation vs. Inhibition of Cellular HDAC Activity

Memory CD4+ T-cells (Leucopaks): Pulse Treatments

Cell-associated vRNA

Class I/II Enzyme Activity

HIV activation (fold above ctrl)

Total HDAC activity (% ctrl)

RMD 40nM
SAHA 1μM
PANO 25nM
A5315 Phase I/II Study (ACTG): RMD in HIV+ Subjects on cART

- Led by Deb McMahon and John Mellors (U Pitt)

- **Study Design**
  - HIV+ Subjects on cART (VL < 50 c/mL)
  - Single Ascending Dose

- **Endpoints**
  - Changes in HIV RNA levels in resting CD4 T cells 24hr post-dose
  - Plasma HIV RNA levels 0 – 48hr post-dose (Single Copy Assay)

<table>
<thead>
<tr>
<th>Dose</th>
<th>RMD</th>
<th>SAHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg/m²</td>
<td>1.7 nM</td>
<td>360 nM</td>
</tr>
<tr>
<td>2 mg/m²</td>
<td>7.0 nM</td>
<td></td>
</tr>
<tr>
<td>5 mg/m²</td>
<td>17.5 nM</td>
<td></td>
</tr>
</tbody>
</table>

*Romidepsin PK is linear up to 24 mg/m²*
RMD Activates HIV Expression at Concentrations Achieved in Human Dosing

Resting CD4+ T-cells (Leucopaks): Pulse Treatments

Cell-associated vRNA

HIV activation (fold Δ)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>RMD 3.5nM</th>
<th>RMD 15nM</th>
<th>RMD 40nM</th>
<th>SAHA 1μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>4%</td>
<td>15%</td>
<td>40%</td>
<td>~200%</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0.5 mg/m² 2.0 mg/m² 5.0 mg/m² >400 mg

:% clin. exposure :equiv. clin. Dose*

*Doses selected for A5315 Study
HDACis and Latent HIV Activation

<table>
<thead>
<tr>
<th></th>
<th>Vorinostat (SAHA (Merck))</th>
<th>Romidepsin (Celgene)</th>
<th>Panobinostat (Novartis)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose</strong></td>
<td>400 mg p.o.</td>
<td>14 mg/m² i.v.</td>
<td>20 mg p.o.</td>
</tr>
<tr>
<td>*<em>Clinical C&lt;sub&gt;max&lt;/sub&gt;</em></td>
<td>360 nM</td>
<td>50 nM</td>
<td>17.5 nM</td>
</tr>
<tr>
<td><strong>In vitro HIV activation EC&lt;sub&gt;50</strong></td>
<td>4,000 nM</td>
<td>4.5 nM</td>
<td>10 nM</td>
</tr>
<tr>
<td><strong>Ex vivo HIV activation (all conditions)</strong></td>
<td>12/36 donors</td>
<td>41/46 donors</td>
<td>2/4 donors</td>
</tr>
<tr>
<td><strong>Ex vivo HIV activation (6-24h cell-associated)</strong></td>
<td>9/11 donors</td>
<td>12/13 donors</td>
<td>2/2 donors</td>
</tr>
</tbody>
</table>

*Based on free drug concentrations
## Summary of HDACis in Clinical Testing

<table>
<thead>
<tr>
<th></th>
<th>Vorinostat SAHA (Merck)</th>
<th>Romidepsin (Celgene)</th>
<th>Panobinostat (Novartis)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Route of Administration</strong></td>
<td>Oral</td>
<td>Intravenous</td>
<td>Oral</td>
</tr>
<tr>
<td><strong>DDIs with ARVs</strong></td>
<td>Minimal</td>
<td>Yes</td>
<td>Minimal</td>
</tr>
<tr>
<td><strong>Ames Test (Mutagenicity)</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Potency to Reactivate HIV (in vitro)</strong></td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td><strong>Human Exposure/EC\textsubscript{50} for HIV Activation</strong></td>
<td>0.1</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td><strong>Ex vivo HIV activation (patient cells)</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>In vivo HIV activation</strong></td>
<td>Yes</td>
<td>???</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Killing of CD4+ T-cells by HIV-specific CTLs
Shan et al., Immunity 2012

We may need strategies to enhance killing of memory T-cells expressing reactivated HIV
Potential Strategies to Kill Cells Expressing Reactivated HIV

- CD8 CTL: Cytolysis
  - Vaccine
- NK cell: Cytolysis
  - TLR7/8 Agonist?
- Bi-specific Ab
- ADC
- mAb mediated:
  - Phagocytosis
  - NK-ADCC
  - Complement

Activation of Latent HIV
Immune Modulators
Toll-like Receptor 7 (TLR7)

- Toll-like receptors (TLR) sense pathogen patterns
- TLR7 detects single-stranded RNA
- TLR7 activation leads to production of type I interferons (IFNα, IFNβ)
- GS-9620: Potent small molecule TLR7 agonist in clinical development
In addition, TLR7 agonists enhance pDC migration to lymphoid tissues and antigen presentation.

TLR7 Agonists Activate CD8+ T-cells and NK Cells in vitro

- **CD8+ T Cell Activation**
- **NK Cell Activation**
- **Functional**

![Graphs showing CD69+ expression and K562 lysis](graphs)

- In addition, TLR7 agonists enhance pDC migration to lymphoid tissues and antigen presentation.
GS-9620 In vivo Studies

- **HBV-infected Chimps**
  - Treatment: 8 weeks
  - vDNA and HBsAg reduction
  - Dose-dependent IFNα increase
  - Activation of B-, T- and NK cells

- **WHV-infected Woodchucks**
  - Treatment: 4 weeks
  - vDNA and WHsAg reduction
  - Induction of anti-WHsAg Abs

- **Healthy Volunteers**
  - Phase I Study
  - SAD

- **HBV-infected Subjects**
  - Phase I Study

---

Lopatin et al, Lanford et al, Menne et al (EASL 2011)
Activate HIV Expression
Romidepsin

Cell-mediated Killing via immune modulation
TLR7 agonist

- Can activation of DCs help prime HIV-specific immune responses?
- Can activated CD8+ CTLs and NK cells help clear cells expressing reactivated HIV?

Study in SIV-infected rhesus macaques on ART underway
HIV Monoclonal Antibodies
Antibody-mediated Effector Functions to Clear Latent Reservoirs?

HIV Activation / Cell Surface Expression of gp120
- gp120 expression levels?

Antibody Recognition
- Binding affinity to HIV gp120?
- Fc receptor recognition?

Immune Clearance
- Effector cell function?
- Tissue/compartment?

ADCC
*(Ab-dependent cellular cytotoxicity)*
- NK cells
- FcgR3a
- peripheral blood

ADCP
*(Ab-dependent cellular phagocytosis)*
- Monocytes + neutrophils
- FcgR2a
- lymph nodes, tissues

ADCD
*(Complement-dependent cytotoxicity)*

Latently-infected CD4+ T cell

Inducing agent

HIV gp120

Anti-gp120 mAb
TLR Agonists Enhance ADCC


VTX-2337 Is a Novel TLR8 Agonist ThatActivates NK Cells andAugments ADCC


Reciprocal Regulation of Activating and Inhibitory FcγReceptors by TLR7/8 Activation: Implications for TumorImmunotherapy

Jonathan P. Butchar, Payal Mehta, Steven E. Justiniano, et al.

2009 British Journal of Haematology, 146, 282–291

Phase II study of a TLR-9 agonist (1018 ISS) with rituximab in patients with relapsed or refractory follicular lymphoma

Jonathan W. Friedberg,1 Jennifer L. Kelly,1 Donna Neuberg,2 Derick R. Peterson,1 Jeffery L. Kutok,2 Rabih Salloum,1 Thomas Brenn,2 David C. Fisher,2 Elizabeth Ronan,2 Virginia Dalton,2 Lynn Rich,1 Diana Marquis,

Cancer Letters 272 (2008) 70–76

Toll-like receptor agonists and invariant natural killer T-cells enhance antibody-dependent cell-mediated cytotoxicity (ADCC)

María Moreno a,b, Berber M. Mol a, Silvia von Mensdorff-Pouilly b, René H.M. Verheijen c, B.Mary E. von Blomberg a, Alfons J.M. van den Eertwegh d

RMD + anti-HIV mAb + TLR7 Agonist?
Therapeutic Vaccines
Picker CMV Vector-based Vaccine

Characteristics: Broad, High Frequency, Durable Effector Memory CD8+ T-Cell Response
Summary

- Three HDACis (vorinostat, panobinostat, romidepsin) are being tested in HIV+ subjects (ART)
  - RMD is more potent, greater max effect and more durable vs. SAHA
  - RMD Induces production of extracellular virions
  - RMD consistently activates HIV ex vivo (N = 46 donors)
  - [RMD] that activate HIV are < [RMD] plasma for CTCL treatment
  - HIV induction correlates with inhibition of HDACs

- TLR7 Agonist GS-9620 activates DCs, CD8+ T-cells and NK cells
  - RMD+TLR7 agonist combo in SIV+ RM (ART) underway

- Determine if anti-gp120 mAbs can kill memory CD4+T-cells expressing reactivated HIV

- CMV-vector based vaccination protects ~ 50% RM from SIV infection
  - Evidence of complete clearance of SIV infection in RM
Acknowledgements

Gilead

♦ Bei Li
♦ Jillian Hattersley
♦ Charlene Kon
♦ MJ Edwards
♦ George Stepan
♦ Helen Yu
♦ Nikos Pagratis
♦ Angela Tsai
♦ Vicki Chiang
♦ George Wei
♦ Gregg Jones
♦ Manuel Tsiang
♦ Michael Graupe
♦ Bing Lu
♦ Jim Zheng
♦ Tiffany Barnes
♦ Anne Chester
♦ Tomas Cihlar
♦ Paul Duatschek
♦ Joe Hesselgesser

U of Pittsburgh

♦ John Mellors
♦ Deb McMahon
♦ Elizabeth Fyne

SAIC-Frederick

♦ Jeff Lifson
♦ Greg Del Prete

Quest Clinical

♦ Jay Lalezari
♦ Michele King