Dasatinib preserves SAMHD1 antiviral activity in CD4+ T cells treated with IL-7
Disclosures

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- Speaker in seminars, conferences and training programs sponsored by
  - Bristol Myers Squibb
  - ViiV Healthcare
  - Gilead Sciences
  - Jansenn Pharmaceutical
The paradigm of latency and reactivation

Resting CD4+ T cell
- Restriction to infection and replication
  - Low CCR5 expression → Entry
  - Low dNTPs levels → Retrotranscription
  - Low ATP levels → Nuclear import
  - Low levels of active transcription factors

Activated CD4+ T cell
- High susceptibility to infection and replication

Infected resting memory CD4+

Mechanisms of generation of latently-infected cells?

Alcamí et al. SAMHD1-IL7
Background. The issue of HIV latency

The mechanisms for the establishment of HIV-1 post-integration latency in quiescent CD4+ T cells are not fully understood.

**Before being killed infected memory CD4 return to a resting state harboring an integrated provirus**

**“Weak” stimuli as chemokines and γC cytokines leads to partial integration allow integration but not viral transcription**

_Vandergeeten G et al. Cytokine Growth Factor Rev. 2012_
In CD4 lymphocytes SAMHD1 is a major element regulating both entry in cell cycle and HIV-1 replication. SAMHD1 is regulated by phosphorylation.

In resting (G0) CD4 lymphocytes SAMHD1 decreases dNTP levels blocking both cell division and HIV-1 retrotranscription.
Hypothesis. γC cytokines and latency

In CD4 lymphocytes γc cytokines (IL2 and IL7) phosphorylate SAMHD1 and relieve restriction to HIV-1 infection but due to their low transcriptional activity would allow latent HIV-1 integration contributing to the generation of viral reservoirs.
Objectives

1. To analyze the role of γC cytokines (IL2 and IL7) in the establishment of latent HIV-1 reservoirs through inactivation of SAMHD1.

2. To assess the potential role of a tyrosine kinase inhibitor – Dasatinib – in the inhibition of SAMHD1 phosphorylation and its potential use as to decrease the reservoir size by increasing SAMHD1 activity.
Methods

Phosphorylation of SAMHD1 at T592 was determined by immunoblotting.

Proviral integration was analyzed by qPCR using TaqMan probes.

Transfection with luciferase vectors under the control of regulatory HIV sequences

Analysis of drugs able to interfere with SAMHD1 phosphorylation.

CD4+ T cells were isolated from PBMCs from healthy donors

+ IL-7 or IL-2 5 days

NL4-3_Renilla
Results (1). SAMHD1 phosphorylation by γc cytokines

- Activation by CD3/CD28 induced SAMHD1 phosphorylation at T592 in CD4+ T cells.

- SAMHD1 phosphorylation is sustained in the presence of IL-2.

- IL-2 and IL-7 phosphorylate SAMHD1 after 3 days of treatment.

Western blot analysis of the kinetics of SAMHD1 phosphorylation in CD4+ T cells treated with different stimuli.
Results (2). Impact of IL2 and IL7 in preintegration steps of HIV

- IL-2 and IL-7 increase retrotranscription and proviral integration.
- IL-7 was more efficient than IL-2 for inducing proviral integration.

CD4+ T cells treated with IL-7 or IL-2 for 5 days, and infected thereafter with a NL4.3-Renilla infectious clone. Reverse transcripts were quantified by RT-PCR 5 hours after infection. Proviral load was assessed after 5 days of infection.
Results (3). Role of chemokines in SAMHD1 phosphorylation and HIV-1 integration in combination with IL2 and IL7

- Treatment with chemokines (CXCL9, CXCL10, CXCL12) did not induce pSAMHD1.

<table>
<thead>
<tr>
<th>t=0</th>
<th>t=5 days</th>
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<tbody>
<tr>
<td>Ø</td>
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<tr>
<td>IL-2</td>
<td>IL-7</td>
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<tr>
<td>CXCL12</td>
<td>CXCL9/10</td>
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</table>

• Simultaneous treatment with CXCL9 and CXCL10 enhanced IL-7/IL2-driven proviral integration, mostly with IL-2.
Results (4). IL2 and IL7 display low transactivation activity

- IL-2 and IL-7 induced low NF-κB and LTR-dependent viral transcription.
- Transactivation of NF-κB and LTR-dependent vectors was not complete as it was fully induced by PMA.
- IL-7 treatment induced latent integration and low basal viral transcription that was increased by PMA treatment.

CD4+ T cells treated with IL-7 or IL-2 for 5d, transfected by Amaxa with LUC expression vectors under the control of NF-κB consensus sites or the HIV LTR. Cultures were treated with PMA for 18 hours before measurement of luciferase activity.

CD4+ T cells were treated with IL-7 for 5 days and infected with NL4-3_Renilla for 5 additional days. Cultures were treated with PMA for 18 hours before measurement of luciferase activity.
Results (5). In vivo trials with IL7

- The exposure of resting CD4+ T cells to cytokines as IL-7 has been associated with an increase in proviral load after in vivo treatment.

Chomont et al. Blood 2013

Katlama et al. ERAMUNE 01. CROI 2013
-10 patients treated with one single dose of IL7 (3, 10 and 30 µg/kg) 
- Frozen PBMCs before and 4 days after treatment were obtained 
- The percent of cells expressing Phosphorylated SAMHD1 in different CD4 subsets was assessed by flow cytometry with a specific monoclonal antibody.

**Table:**

<table>
<thead>
<tr>
<th>CD4 Subset</th>
<th>Day 0</th>
<th>Day 4</th>
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<tbody>
<tr>
<td>$T_{NA}$</td>
<td>6.0 ± 1.1</td>
<td>8.9 ± 1.5</td>
</tr>
<tr>
<td>$T_{CM}$</td>
<td>10.3 ± 2.0</td>
<td>18.3 ± 1.9</td>
</tr>
<tr>
<td>$T_{EM}$</td>
<td>9.8 ± 2.0</td>
<td>16.8 ± 1.9</td>
</tr>
<tr>
<td>$T_{TD}$</td>
<td>4.1 ± 1.0</td>
<td>9.3 ± 3.3</td>
</tr>
</tbody>
</table>
Results (6). Tyrosine kinase inhibitors decrease HIV-1 infection and inhibit SAMHD1 phosphorylation by immune stimuli

Dasatinib inhibits both a VSV-pseudotyped and wild type HIV at the same IC50 suggesting a target beyond viral entry.
Results (7) Dasatinib inhibits retrotranscription and viral infection in IL7-treated lymphocytes

Dasatinib inhibits HIV-1 retrotranscription and decreases viral replication in CD4 lymphocytes activated with IL7.

A. LTCD4+ IL-7

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>∅</td>
<td>75 nM</td>
</tr>
<tr>
<td>pSAMHD1</td>
<td></td>
</tr>
<tr>
<td>Total SAMHD1</td>
<td></td>
</tr>
</tbody>
</table>

B. LTCD4+ IL-7

- Untreated
- Dasatinib 75nM

Copy no retrotranscripts

- Early RT
- Late RT

C. LTCD4+ IL-7

- Untreated
- Dasatinib 75 nM
Results (8) In vivo treatment with Dasatinib decreases SAMHD1 phosphorylation “ex vivo”

- CD4+ T cells from patients on chronic treatment for Dasatinib showed lower SAMHD1 phosphorylation after activation with PHA/IL-2 for 48 hours.
Results (9) In vivo treatment with Dasatinib decreases susceptibility to HIV-1 infection “ex vivo”

- CD4+ T cells from patients on chronic treatment for Dasatinib appear less susceptible to HIV-1 infection in vitro.

Analysis by qPCR of the proviral integration (A) and production of Renilla (B) in CD4+ T cells from three patients on treatment with Dasatinib for several years, compared to 7 healthy controls. PBMCs were activated with PHA/IL-2 48 hours before infection with NL4.3-renilla replication-competent viral clone for five days.
Proposed hypothesis

First step
- No activation
  - Full restriction
  - SAMHD1
- Resting state

Second step
- Homeostatic activation IL-2/IL-7
  - Retrotranscription and integration
  - Low transcription
  - SAMHD1
- Suboptimal activation

Third step
- Full replication
  - Full activation and viral transcription
  - Antigen
  - NF-κB
  - NF-AT
  - AP1

Resting state
- Naive CD4+ T cell
- Post-integration latency
- Low ongoing replication

Suboptimal activation
- TK inhibitors
1. In our models, SAMHD1 regulation plays a central role in the establishment of viral reservoirs and represents a major target for therapeutic intervention.

2. Treatment with γc-cytokines IL-2 and IL-7 enhanced phosphorylation of SAMHD1 “in vitro” and favor latent HIV-1 proviral integration in CD4 lymphocytes.

3. In vivo treatment with IL7 phosphorylates SAMHD1, mainly in central memory CD4 lymphocytes.

4. IL7 can increase the size of the viral reservoir not only by homeostatic proliferation but through Inhibition of SAMHD1-mediated restriction leading to increased susceptibility to HIV-1 infection.

5. Tyrosine kinase inhibitors protects SAMHD1 from IL7-mediated phosphorylation and could be used to preserve its antiviral function.

6. Treatment with Lck inhibitors could be particularly useful during acute infection to decrease cellular activation and to enhance the restriction capacity of SAMHD1.
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- BACK UP SLIDES
Resting PBMCs were infected with replication competent viral clone (NL4-3_Renilla) loaded or not with Vpx, in the presence or absence of Dasatinib. These cells were activated with PHA/IL-2 for 5 days and the production of Renilla was then analyzed.
Resting PBMCs were infected for 5 hours with replication competent viral clone (NL4-3_Renilla) loaded or not with Vpx, in the presence or absence of Dasatinib. These cells were then treated with PHA/IL-2 for 5 days and the production of Renilla was then analyzed.

PBMCs from three controls and one patient treated with Dasatinib were activated for 5 days with IL7 before infection with a replication-competent viral clone (NL4.3-renilla) loaded or not with Vpx for five days and the production of Renilla was then analyzed.
Results (3). Role of chemokines in SAMHD1 phosphorylation and HIV-1 integration in combination with IL-2 and IL-7

- Treatment with chemokines (CXCL9, CXCL10, CXCL12) did not induce pSAMHD1.
  \[ t=5 \text{ days} \]

<table>
<thead>
<tr>
<th></th>
<th>t=0</th>
<th>CXCL12</th>
<th>CXCL9/10</th>
<th>CD3/CD28</th>
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<tbody>
<tr>
<td>∅</td>
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<td></td>
</tr>
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- Simultaneous treatment with CXCL9 and CXCL10 enhanced IL-7/IL2-driven proviral integration, mostly with IL-2.
RESULTS (2)

- IL-2 and IL-7 phosphorylate SAMHD1 after 3 days of treatment.

- pSAMHD1 was quite stable as it persisted for 48h after removing the stimuli from the culture medium.

WB of total protein extracts from CD4+ T cells treated with IL-7 or IL-2 for 5 days

WB of total protein extracts from CD4+ T cells treated with IL-7 or IL-2 for 5 days and then depleted of both stimuli and incubated for 48 hours
Results (3) DASATINIB INTERFERES WITH HIV-1 CYCLE FROM RETROTRANSCRIPT

Analysis by qPCR of HIV-1 replication cycle in CD4+ T cells treated with Dasatinib before adding PHA/IL-2 for 48 hours.

Analysis of viral transcription in CD4+ T cells treated with Dasatinib before adding PHA/IL-2 for 48 hours.
RESULTS (4)

- Treatment with chemokines (CXCL9, CXCL10, CXCL12) or TNFα did not induce pSAMHD1.
- Expression of total SAMHD1 was not modified by any stimulus.
Results (5). IL7 treatment phosphorylates SAMHD1 “in vivo”

- 10 patients treated with one single dose of IL7 (3, 10 and 30 µg/kg)
- Frozen PBMCs from before and 4 days after treatment were obtained
- The percent of cells expressing SAMHD1 was assessed in different CD4 subsets by flow cytometry with a specific monoclonal antibody.
Results (8) In vivo treatment with Dasatinib decreases SAMHD1 phosphorylation “ex vivo”

- CD4+ T cells from patients on chronic treatment for Dasatinib showed lower SAMHD1 phosphorylation after activation with PHA/IL-2 for 48 hours.

- CD8+ T cells from these patients do not loose IFN responses to specific antigens (CMV/EBV/Influenza)
Objective 2. To assess the potential role of a tyrosine kinase inhibitor – Dasatinib – in the inhibition of SAMHD1 phosphorylation and its potential use as to decrease the reservoir size by increasing SAMHD1 activity.

Wang et al., Front Immunol. 2012

Coiras et al. Biochem Pharmacol 2015
Conclusion

I.P. Alcami et al. SAMHD1-IL7

Resting CD4+ T cell

SAMHD1

IL-2/IL-7

Suboptimal activation

Post-integration latency

Low ongoing replication

TcR-mediated activation

Proviral integration

Full activation

Full viral replication

SAMHD1

IL-2/IL-7

P

P

TcR-mediated activation

Proviral integration

Full activation

Full viral replication

SAMHD1

P
Proposed hypothesis: three steps

First step
- No activation
- Full restriction
- SAMHD1

Second step
- Homeostatic activation IL-2/IL-7
- Retrotranscription and integration
- Low transcription
- SAMHD1

Third step
- Full activation and TcR activation
- Full replication
- NF-κB
- NF-AT
- AP1

Resting state
- Naive CD4+ T cell

Suboptimal activation
- Post-integration latency

Full activation and viral transcription
- Low ongoing replication
- Antigen
- Epitope
RESULTS (2)

- IL-2 and IL-7 phosphorylate SAMHD1 after 3 days of treatment.

- pSAMHD1 was quite stable as it persisted for 48h after removing the stimuli from the culture medium.

WB of total protein extracts from CD4+ T cells treated with IL-7 or IL-2 for 5 days

WB of total protein extracts from CD4+ T cells treated with IL-7 or IL-2 for 5 days and then depleted of both stimuli and incubated for 48 hours
Background: γc cytokines (IL-2, IL-7)

- The exposure of resting CD4+ T cells to cytokines as IL-7 has been related to increased proviral load after in vivo treatment.

- Both IL-2 and IL-7 are bad inducers of HIV-1 reactivation from latency.

Katlama et al. ERAMUNE 01. CROI 2013


Chomont et al. Blood 2009

Alcamil. Unpublished
Results (3). DASATINIB INHIBITON OF INFECTION IS OVERCOME BY VPX

(A) Analysis of early and late retrotranscription (RT) in resting PBMCs infected for 5 hours with NL4-3_Renilla with or without SIVsm Vpx, in the presence or absence of Dasatinib. (B) These cells were then treated with PHA/IL-2 for 5 days and the production of Renilla was then analyzed.
Background: \( \gamma c \) cytokines (IL-2, IL-7)

- The exposure of resting CD4+ T cells to cytokines as IL-7 has been related to increased proviral load after in vivo treatment.

Katlama et al. ERAMUNE 01. CROI 2013
Análisis por WB de SAMHD1 total o fosforilado en T592 en extractos totales de LTCD4 + PHA/IL-2 48h, infectados con NL4-3_Renilla + pcDNA3 o NL4-3_Renilla + Vpx (por duplicado) durante 5 días.
ACTG 5214
N=18 patients treated with one single dose of IL7 at 3-10-30 and 60 μg/Kg, N=6 placebo

(Seretti et al. Blood 2009)
ACTG 5214
N=7 patients treated with one single dose of IL7 at 3-10-30 and 60 μg/Kg, N=3 placebo

(Chomont et al. Blood 2013)
Dasatinib interferes with HIV-1 replication

- Dasatinib interferes with HIV-1 cycle from retrotranscription.
- Infection with virions carrying Vpx abrogates the inhibitory effect of Dasatinib.
Dasatinib interferes with HIV-1 replication

- CD4+ T cells from patients on chronic treatment for Dasatinib showed lower SAMHD1 phosphorylation after activation with PHA/IL-2 for 48 hours.
- CD4+ T cells from these patients appear less susceptible to HIV-1 infection in vitro.