

# Screening for Inhibitors of CD4-PLSCR1 Interaction, a New Target for Therapy of HIV-1 Infection

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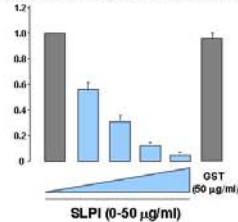
## 1 - The interaction between the CD4 receptor and the phospholipid scramblase 1 (PLSCR1) may constitute a new target to block HIV-1 entry

Recent work in the laboratory (B. Py, 2009, PLoS one in press) showed that SLPI-like inhibitors of the CD4-PLSCR1 interaction could be useful in a new antiviral strategy

- ✓ CD4 receptor interacts with PLSCR1, an enzyme controlling the movements of phospholipids at the plasma membrane
- ✓ PLSCR1 is able to bind SLPI (Secretory Leukocyte Protease Inhibitor), a peptide secreted in all mucous liquids and displaying a powerful antiviral activity against HIV-1
- ✓ SLPI antiviral activity may be related to the disruption of the CD4-PLSCR1 interaction

### Recombinant SLPI inhibits HIV-1 replication in primary T lymphocytes

Virus replication in HIV-1 infected cells (ratio of p24 production in medium over control)

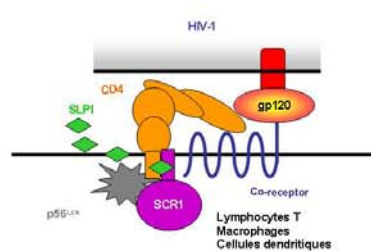


### Disruption of the CD4-PLSCR1 interaction by SLPI

Western blot of bound proteins with anti-CD4  
GST or GST-PLSCR1 was incubated with lysates from Jurkat positive T cells in presence of GST-SLPI or GSTARF1 (control)



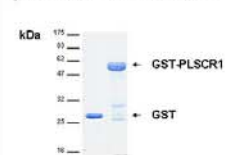
### Proposed mechanism for SLPI antiviral activity



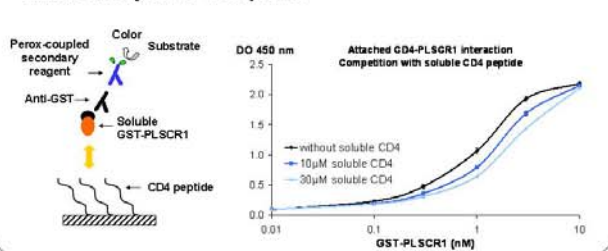
## 2 - An ELISA test was designed to measure the potential inhibitory effect of compounds on the CD4-PLSCR1 interaction

Recombinant human GST-PLSCR1 and test compounds are incubated with an immobilized chemically-synthesized peptide (AERMSQIKRLLSEKKTQAQAPHRFQKTCSPI) derived from the C-terminal cytoplasmic domain of CD4

### E. coli-expressed and purified GST-PLSCR1

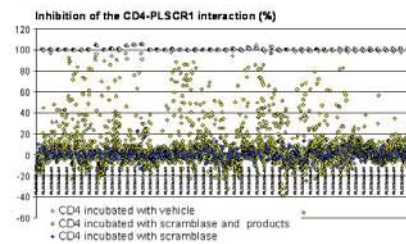


### ELISA assay in 96-well plates



## 3 - A library of "privileged structures" was screened to find inhibitors of the CD4-PLSCR1 interaction using the ELISA assay

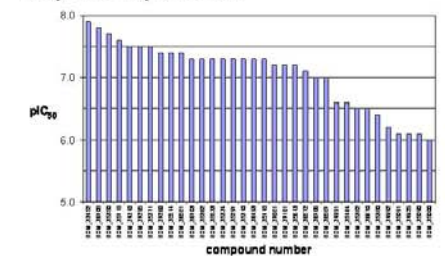
4000 compounds were screened at 1 µM (Z': 0.82)



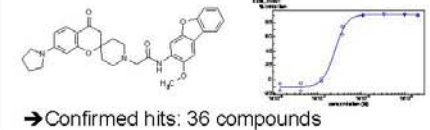
inhibition (%)	nb of compounds	% library
>30	236	5.90%
>50	104	2.60%
>80	24	0.60%
>90	3	0.08%

→ Primary selected hits: 36 compounds (the 31 more active & 5 giving around 50% inhibition)

Primary hits were retested in dose response experiments



- ✓ Measured IC<sub>50</sub> values range from 30 nM to 1 µM
- ✓ As an example BDM-34991 inhibits the CD4-PLSCR1 interaction with an IC<sub>50</sub> value of 250 nM



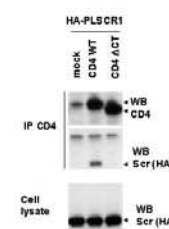
→ Confirmed hits: 36 compounds

## 4 - Conclusion and ongoing studies

- ✓ Several families of non-peptide disruptors of the CD4-PLSCR1 interaction were discovered
- ✓ According to their IC<sub>50</sub> values and structural considerations, 10 hits have been selected for further evaluation in cell-based interaction and virological assays

### Co-precipitation of CD4 and PLSCR1 proteins

293T cells expressing HA-tagged PLSCR1 (lower panel), Cell lysate in combination with wild-type CD4 (WT) or a mutant of CD4 deleted of its cytoplasmic domain (DCT) will be lysed, incubated with the test compounds and the CD4 forms will be precipitated with anti-CD4. Precipitates will be then analyzed by Western blot with anti-CD4 (upper panel) or anti-HA (middle panel).



### Virological assay

The virus particles, that may be pseudotyped with different types of HIV-1 envelopes, and containing a transgene coding for GFP, are produced by transfection of cells. After incubation with test compounds, the virus/compound mixture will be used for infection of various cell types expressing CD4. Virus infectivity is analyzed later by evaluating the percentage of cells expressing GFP by flow cytometry.

