Publishable Executive Summary

1. Objectives

Despite the success of highly active antiretrovirals to control HIV replication in infected patients, at least in countries that can afford these treatments, new drugs are still needed. Widely used drugs mainly target two viral enzymes, reverse transcriptase and protease. However, about 20% of patients cannot tolerate antiviral cocktails in the short term, and long-term treatments are often associated with severe side effects. There is also increasing concern about the spreading of drug-resistant HIV variants.

Our aim is to **identify lead compounds that could impact HIV through new mechanisms.** Academia experts in virology and cellular biology have joined forces with antiviral-research specialists and pharmacologists, in order to perform **anti-HIV high-throughput screening (HTS) assays**. We have defined one unexploited viral target, for which there are no available inhibitors: The critical step of viral release from the cell. This novel target has been chosen because important recent discoveries have shed new light into the molecular mechanisms of virus budding, thereby rendering this critical step in HIV life cycle a feasible target for drug development.

We have designed one cell-based assay, which does not require the use of infectious virus, allowing the screening of libraries of chemicals. As a proof of concept, we have screened 2,000 compounds, and were able to identify one interesting hit. In secondary analysis with infectious HIV, this compound display very little antiviral activity. Our next aim is to extend the screening to an higher number of compounds (two libraries of 20,000 and 4,000 compounds, should be screened). We will also document further the activity of he first hit. On a more fundamental point of view, we are studying the mechanisms of HIV-1 assembly and transfer through cell-to-cell contacts.

We hope that the screening assay will allow the identification of hits or lead compounds, which could in a further step be improved by using a classical drug design approach.

2. List of Partners

Partner 1. Olivier Schwartz (Institut Pasteur, Paris, France)
Partner 2. Kalle Saksela (Institute of Tempere, Finland)
Partner 3. Barbara Muller (University Clinic, Heidelberg, Germany)
Partner 4. Maurizio Federico (Istituto Superiore di Sanita, Rome, Italy)
Partner 5. Marcel Hibert (Université Louis Pasteur, Strasbourg, France)

Scientific coordinator: Olivier Schwartz, email: schwartz@pasteur.fr
Coordinator organization name: Institut Pasteur

3. Results achieved so far

During the two years of this STREP (corresponding to WP1 and WP2 of the proposal), the resources were used essentially as planned for all contractors. There were no major delays in the experiments, and most of the intended work has been performed. WP1 was aimed at designing two assays (one cell-based assay and one cell-free, protein/protein interaction assay) allowing the screening of libraries of compounds, targeting a unexploited step of the viral cycle, the release of viral particles from the infected cell. Various strategies were designed, in order to select optimal method(s) to perform each assay.

Regarding the cell-based assay, we have designed both transient and stable systems to express HIV-like particles (VLPs) and we have tested various techniques to measure release of the VLPs in the supernatants. We have selected one transient system and we are in the process of obtaining a stable cell system to reliably measure viral release. Viral release can be measured with a fluorescent readout for the screening. We have already screened 2,000 compounds with the transient system of viral release (WP2). This screening will be extended to other library of compounds in the near future.

With the cell-free system of screening, aimed at inhibiting protein/protein interactions, we have designed a test allowing the detection of the binding of one HIV protein, Gagp6, to one cellular protein, Tsg101. However, this system was not sensitive enough to perform a screening assay. As a complementary strategy, we have selected two other proteins involved in viral replication (HIV protein Nef and its cellular interactor Hck), which have a strong affinity for each other (better than that of Gagp6 with Tsg101). We have performed a screening of the library of chemicals (4,000 compounds) with this system. This screening will also be extended to other library of compounds in the near future.

The compounds selected in each screening were further analyzed in secondary assays. Hits inhibiting the release of fluorescent VLPs were tested with infectious HIV. Only one compound displayed a minor antiviral activity, and this compound is currently being analyzed more precisely. Hit compounds inhibiting Nef-Hck interactions were tested for their capacity to inhibit Nef function in T lymphocytes (CD4 and MHC-I down-regulation). Unfortunately, none of the selected compounds inhibited these functions of Nef.

We hope to identify interesting molecules by increasing the number of compounds tested in the two screening assays.

We have published various papers on HIV assembly, and on various experimental systems to study release of fluorescent and non-fluorescent VLPs We have also pursued our basic research work aimed at understanding the basic mechanisms of replication of HIV and other retroviruses in various experimental systems, as detailed in the publication list below.

4. Publications and patents 2005-2007 (selected).

Delebecque F, Suspène R, Calattini S, Casartelli N, Saïb A, Froment A, Wain-Hobson S, Gessain A, Vartanian J.P, Schwartz O. Restriction of Foamy Viruses by APOBEC cytidine deaminases, **J. Virol.** 2006 ; 80 : 605-14

Nobile C, Petit C, Moris A, Skrabal K, Abastado JP, Mammano F, Schwartz, O. Covert HIV replication in dendritic cells and in DC-SIGN-expressing cells promotes long-term transmission to lymphocytes. **J Virol** 2005 ; 79 :5386-5399

Esnault C, Heidmann O, Delebecque F, Dewannieux M, Ribet D, Hance AJ, Heidmann T, Schwartz O. APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses. **Nature.** 2005;433:430-433

Vincent, P, Priceputu, E, Kay, D, Saksela, K, Jolicoeur, P, and Hanna, Z: Activation of PAK2 and its association with Nef is conserved in murine cells, but is not sufficient to induce an AIDS-like disease in CD4C/HIV transgenic mice. **J. Biol. Chem.**, 2006; **281**:6940-54.

Kärkkäinen, S., Hiipakka, M., Wang, J.H., Kleino, I., Vähä-Jaakkola, M., Renkema, G.H., Liss, M., Wagner, R., Saksela, K.: Identification of preferred protein interactions via phage-display of the human SH3 proteome. **EMBO Reports**. 2006; **7**: 186-191

Sticht J, Humbert M, Findlow S, Bodem J, Müller B, Dietrich U, Werner J, Kräusslich HG. (2005) A peptide inhibitor of HIV-1 assembly in vitro. **Nat Struct Mol Biol**. 12:671-7

Olivetta E, Pietraforte D, Schiavoni I, Minetti M, Federico M, Sanchez M. HIV-1 Nef regulates the release of superoxide anion from human macrophages. **Biochem** J. 2005, Sep 1;390(Pt 2):591-602.

Peretti S, Schiavoni I, Pugliese K, Federico M. Cell Death Induced by the Herpes Simplex Virus-1 Thymidine Kinase Delivered by Human Immunodeficiency Virus-1-Based Virus-like Particles. **Mol Ther**. 2005 Dec;12(6):1185-96. Epub 2005 Aug 10.

Peretti S, Schiavoni I, Pugliese K, Federico M. Selective elimination of HIV-1 infected cells by Env-directed, HIV-1 based Virus Like Particles. **Virology**. 2006 Feb 5;345(1):115-26

Olivetta E. & Federico M. HIV-1 Nef protects human monocyte-derived macrophages from HIV-1 induced apoptosis. **Exp. Cell Res** 2006 Apr 1;312(6):890-900

Welsch S, Habermann A, Jäger S, Müller B, Krijnse-Locker J, Kräusslich HG. Ultrastructural analysis of ESCRT proteins suggests a role for endosome-associated tubular-vesicular membranes in ESCRT function. **Traffic.** 2006 Nov;7(11):1551-66.

Lampe M, Briggs JA, Endress T, Glass B, Riegelsberger S, Kräusslich HG, Lamb DC, Bräuchle C, Müller B. Double-labelled HIV-1 particles for study of virus-cell interaction. **Virology.** 2006 Nov 8; [Epub ahead of print]

Muratori, C., D'Aloja, P., Superti, F., Tinari, A., Sol-Foulon, N., Sparacio, S., Bosch, V., Schwartz, O. and Federico, M. (**2006**) Generation and characterization of a stable cell population releasing fluorescent HIV-1-based Virus Like Particles in an inducible way. **BMC Biotechnol**, **6**, 52.

Sol-Foulon, N., Sourisseau, M., Porrot, F., Thoulouze, M.I., Trouillet, C., Nobile, C., Blanchet, F., di Bartolo, V., Noraz, N., Taylor, N., Alcover, A., Hivroz, C. and Schwartz, O. (**2007**) ZAP-70 kinase regulates HIV cell-to-cell spread and virological synapse formation. **EMBO J**, **26**, 516-526.

Sourisseau, M., Sol-Foulon, N., Porrot, F., Blanchet, F. and Schwartz, O. (**2007**) Inefficient human immunodeficiency virus replication in mobile lymphocytes. **J Virol**, **81**, 1000-1012.