

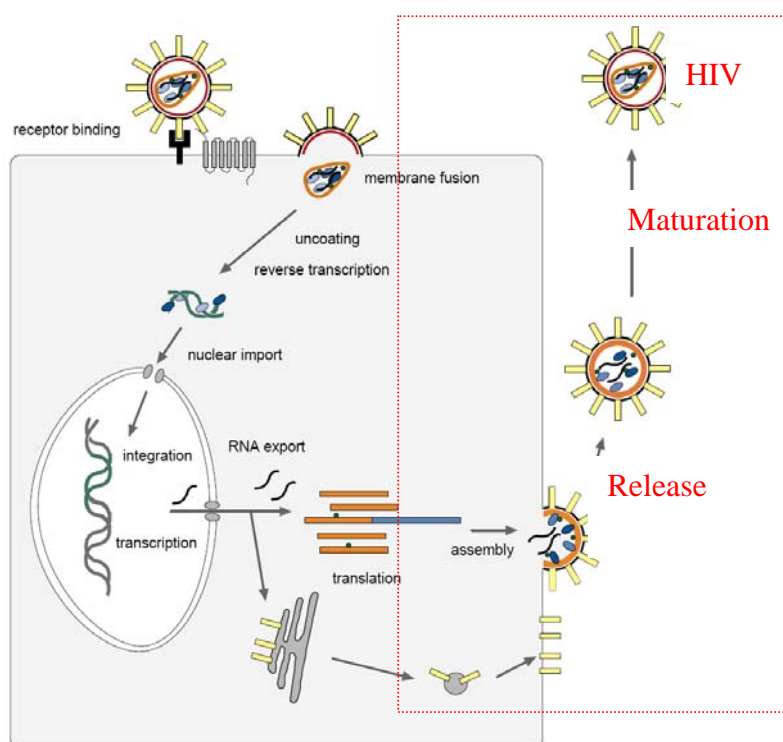
## 1. Final publishable summary report

**Title:** Targeting assembly of infectious HIV particles (FP7-HEALTH-201095)

**Acronym:** HIV-ACE

**Starting date:** 01/03/2008

AIDS is one of the major life-threatening infectious diseases in the world today, with over 33 million people living with the virus and 65 million people infected since the beginning of the epidemic. A constant supply of novel antiretrovirals (ARVs) is needed to respond to the limitations of current drugs. Over thirty different treatments have been developed and approved since the first drug was made available in 1987. This currently approved therapeutic arsenal against HIV comprises ARVs blocking all major steps of the HIV replication, except for particle assembly and budding. Members of HIV-ACE were instrumental in recent breakthroughs concerning virus assembly and Envelope incorporation into virions. Hence the goal of the consortium is to translate progress in understanding the mechanisms involved in HIV-1 capsid assembly, budding and Env incorporation into innovative anti-retroviral drugs (ARV)(Figure 1).



**Figure 1:** Replication cycle of Human immunodeficiency virus. The red square focuses on the assembly, Env incorporation, release and maturation steps of the HIV replication cycle.

On the basis of novel and fully validated targets, the objectives of HIV-ACE are: i) Assay development, drug screening and pre-clinical development of small molecule inhibitors of Capsid assembly and Env incorporation, up to the stage of Early Drug Candidates with acceptable toxicity profile determined by ADME/Tox studies (WP1); ii) Elucidation of 3D structures of these validated targets and rational drug design guided by molecular modeling and docking of inhibitors (WP2); iii) validation and exploitation of the HIV-susceptible transgenic rat model to allow preclinical *in vivo*-evaluation of novel drug candidates from HIV-ACE (WP3); iv) Elucidation of the mechanisms responsible for activity of the validated inhibitors, and discovery/validation of novel targets in the budding pathway of HIV-1 (WP4).

The ordered assembly of infectious viral particles and the release of progeny virions from the host cell are crucial steps in virus replication. In the case of HIV, this involves the assembly of a protein capsid protecting the viral genome, the envelopment of the capsid by a lipid membrane which buds off from the membrane of the producing cell, and the incorporation of virus glycoprotein into this lipid envelope (Figure 1). The aim of WP1 was to identify small molecule inhibitors which interfere either with capsid assembly, viral budding or glycoprotein incorporation, respectively, and thereby inhibit HIV replication. In the first step, partners 1, 2 and 5 (partners' name at the end of the article) have established different *in vitro* assay systems suitable for medium to high throughput screening and have employed these systems to screen large libraries of small molecule compounds (Hermle *et al.*, BMC Biotechnology 2010<sup>1</sup>). These efforts resulted in the identification of small molecules specifically interfering with HIV CA:CAI interaction, with HIV Env:TIP47 interaction, or with HIV particle release in tissue culture, respectively. These initial hits were subsequently characterized with respect to physicochemical properties, cytotoxicity and interaction with the viral protein of interest. Complementary cell biological and virological studies were performed in order to elucidate their mechanism of action. Antiviral activity of the compounds was confirmed by measuring their effect on HIV replication in tissue culture. Starting from initial hits with antiviral potential *in vitro*, iterative cycles of medicinal chemistry and inhibitor testing were performed by partners 1, 2, 5, and 7 in order to improve the inhibitory efficacy and the selectivity index of the respective compounds. While the antiviral potential of release inhibitors selected in tissue culture, as well as of inhibitors of Env:TIP47 interaction proved to be limited in these studies, two modified inhibitors of HIV capsid assembly with a significantly improved selectivity index as compared to the initial hits have entered ADME/Tox studies in mice, which will guide their further development. If successful, this would yield anti-HIV drugs acting through a mechanism different

from that of all currently available antiretrovirals. Partner 2 and 7 are currently preparing a joint patent application for protection of intellectual property through the subsequent stages of development.

The aim of the Rational Drug Design task force (WP2) was to elucidate the three-dimensional structures of potential targets validated in WP1, and to propose, through a rational drug design approach and the determination of target/ligand complex structures, new potential inhibitors molecules. This work package was delegated to partners 1, partners 2 and partners 6. Previous to this project, the crystal structure of the C-terminal domain of the HIV-1 capsid protein (CCA) was determined in complex with a capsid-assembly inhibitor (CAI) peptide. This peptide had been identified by partner 2, and the structure was done in collaboration with partner 6. Under WP2 (Rational Drug Design), *in vitro* and virological analyses carried out by partner 2 on mutated virus on capsid residues involved in CAI binding site allowed the characterization of the residues forming a reactive groove crucial for the capsid assembly and maturation. By a crystallographic approach, Partner 6 determined the structures of C-CA mutants alone or in complex with the CAI peptide (Bartonova *et al.*, J. Biol. Chem. 2008<sup>2</sup>). The structures of complexes between C-CA and CAI peptide showed that the peptide inhibited assembly not only by steric hindrance, but also by altering the CCA dimerization interface. Because small peptides are in general not viable as drugs, a screening of chemical compounds was done by partner 7 to identify putative drugs that bind to this pocket. Six competitors of the inhibitory peptide, obtained from partner 7, were tested in crystallization. In one case, crystals were obtained with the ligand positioned in the pocket. Additional work by WP2 partners is now needed to improve crystal quality and solve the structure of these complexes.

Under WP3, the suitability of the multi-transgenic rat model of HIV infection was demonstrated for testing late-phase drugs by means of a successful proof-of-principle validation of a prototypic protease inhibitor *in vivo*. Furthermore, remaining limitations to highly efficient HIV-1 replication in rat cells were identified and further characterized (Goffinet, Schmidt *et al.* J. Virol 2010a). <sup>3</sup>In particular, the potent and Vpu-insensitive restriction factor rat CD317 imposes a relevant barrier to HIV-1 release in this species (Goffinet *et al.*, Cell Host Microbe 2009<sup>4</sup>). The mechanism by which Vpu counteracts CD317 in human cells was investigated in some detail and identified a subversion of intracellular trafficking of recycling and newly synthesized CD317/BST2 molecules as the key effector function of Vpu to downregulate the restriction factor from the plasma membrane

(Goffinet, Homann, et al. <sup>5</sup>J. Virol 2010b; Erikson et al. PNAS 2011<sup>6</sup>). Additional works complete the discoveries done by the consortium on CD317/BST2 restriction factor. Partner 3 in WP4 has shown that viruses related to HIV-1 that lack the *vpu* gene are able to overcome CD317/BST2 by a new activity encoded by SIV *env* (Gupta et al. PNAS 2009<sup>7</sup>). Moreover, two studies from Partners 1 and 3 report the role of the ESCRT machinery and the endocytic pathway in the mechanism by which Vpu counteracts CD317/BST2 and promotes HIV-1 dissemination (Caillet, Janvier *et al.*, PloS Pathogens 2011<sup>8</sup>). This knowledge now allows us to either introduce additional genetic changes in the host or evolve a rat CD317-targeting Vpu to overcome this limitation in rats.

Overall, this work demonstrates that novel antiviral compounds targeting steps in the HIV-1 replication cycle from entry, over reverse transcription and integration to virion egress can in principle be evaluated in this readily accessible, immunocompetent small animal model. Furthermore, strategies to further optimize replication in this animal model by overcoming the rat CD317 restriction have been designed.

The overall objectives of WP4 (Partners 1, 3 and 9) were to obtain fundamental information concerning the structure and function of host factors that are required for specific aspects of HIV-1 assembly, including the interaction between TIP47 and HIV-1 Env. Such information was obtained and will be critical to guide future development of drugs inhibiting this interaction that is critical to HIV-1 replication. Additionally, the role of TIP47 was confirmed and characterized in macrophages, one of the important cell types of the immune system that is targeted for infection by HIV-1 (Bauby *et al.* Traffic 2010<sup>9</sup>). Other goals of the project included attempts to identify new host factors that regulate the production of infectious HIV-1. Several Rab proteins important generally for transport within cells were screened and Rab7a was found to play an important role in the formation of fully infectious virion particles and needed for one of the viral accessory proteins, Vpu, to counteract CD317/BST2 restriction factor and produce new virion particles (Caillet *et al.* Plos Pathogens 2011<sup>10</sup>). Rab7a, then, becomes a potential new target for inhibitors that block HIV-1 virion assembly. Additionally it was shown that the drug cyclosporine has a specific inhibitory effect on HIV-1 virion particle production and blocks the incorporation of the Env proteins essential for infectivity (Sokolskaja *et al.* J. Virol 2010<sup>11</sup>; Bernasconi *et al.*, Plos One 2010<sup>12</sup>). This is the starting point for development of new HIV-1 inhibitors. Finally, number of additional fundamental discoveries made by the WP4 might be exploited in the future to inhibit HIV-1 assembly. These include the discovery of a discrete compartment within macrophages where HIV-1 assembles new virions (Pelchens-Mathews *et al.*, Traffic 2011 In press<sup>13</sup>) and the finding that the

same transport pathway, the ESCRT machinery, that is required for HIV-1 virions to assemble also regulates a natural, cellular inhibitor of HIV-1 release known as CD317/BST2 (Janvier *et al.*, PloS Pathogens 2011).

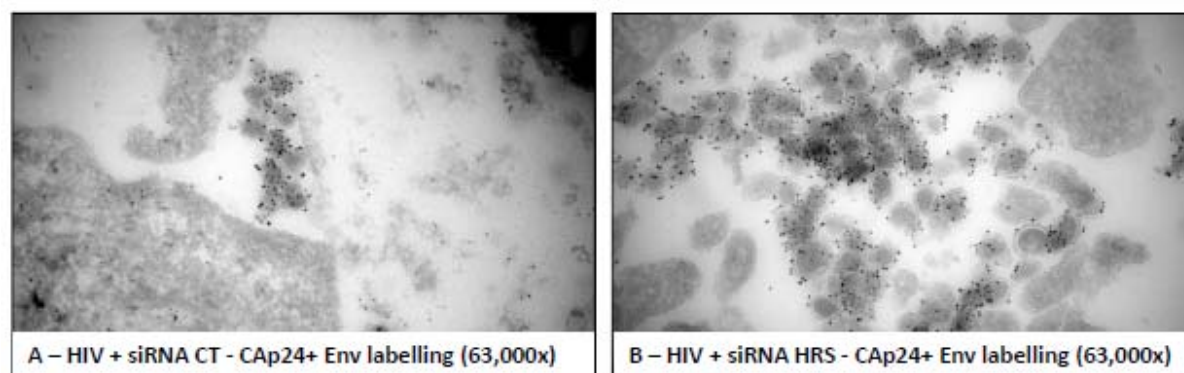


Figure 2: Immuno-Electron microscopic images from sections of HeLa cells infected with HIV-1 and transfected with control siRNA (CT) (A) or siRNA down-regulated HRS (B) expression. The CAP24 and Env components of HIV particles were labelled with gold particles. Depletion of HRS, a component of the ESCRT machinery, induces the accumulation of large aggregates of mature HIV-1 virions at the cell surface and prevents their release into the supernatant. (Janvier *et al.*, Plos pathogens 2011).

Systems have also been developed to allow the direct observation of the assembly of individual HIV virions in live cells, which enables us to measure kinetics of intracellular HIV assembly and the interaction with cellular proteins involved in HIV release (Ivanchenko *et al.*, PloS Pathogens 2009<sup>14</sup>; Eckhardt *et al.* PLoS One 2011<sup>15</sup>; Baumgärtel *et al.*, Nature cell biology 2011<sup>16</sup>). This system is being used for further characterization of assembly inhibitory compounds.

In conclusion, HIV-ACE consortium has been instrumental for major, recent breakthroughs concerning HIV capsid assembly, BST-2 restriction, the characterization of HIV assembly in primary macrophages and the improvement of transgenic rat model. The effort of the consortium also demonstrated that it is possible to discover new HIV inhibitors based on completely different mechanism of action to currently available drugs, and on inhibition of protein-protein interactions instead of the more classical approach of inhibition of the catalytic activity of viral enzymes.

**Key words:** AIDS/HIV/ Capsid assembly / Env incorporation / antiretrovirals / screening / HIV budding / Medicinal chemistry / Virology / Cell biology / Drug discovery / Drug development

**HIV-ACE web-site:** [www.hiv-ace.eu](http://www.hiv-ace.eu)

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<sup>1</sup> A simple fluorescence based assay for quantification of human immunodeficiency virus particle release. PMID: 20406458

<sup>2</sup> Residues in the HIV-1 capsid assembly inhibitor binding site are essential for maintaining the assembly-competent quaternary structure of the capsid protein. PMID: 18772135

<sup>3</sup> Endogenous CD317/Tetherin limits replication of HIV-1 and murine leukemia virus in rodent cells and is resistant to antagonists from primate viruses. PMID: 20702620

<sup>4</sup> HIV-1 antagonism of CD317 is species specific and involves Vpu-mediated proteasomal degradation of the restriction factor. PMID: 19286137

<sup>5</sup> In vivo expression profile of the antiviral restriction factor and tumor-targeting antigen CD317/BST-2/HM1.24/tetherin in humans. PMID: 21808013

<sup>6</sup> In vivo expression profile of the antiviral restriction factor and tumor-targeting antigen CD317/BST-2/HM1.24/tetherin in humans. PMID: 21808013

<sup>7</sup> Simian Immunodeficiency Virus envelope glycoprotein counteracts tetherin/BST-2/CD317 by intracellular sequestration. PMID: 19864625

<sup>8</sup> The ESCRT-0 component HRS is required for HIV-1 Vpu-mediated BST2/Tetherin down-regulation. PMID: 21304933

<sup>9</sup> TIP47 is required for the production of infectious HIV-1 particles from primary macrophages. PMID:20070608

<sup>10</sup> Rab7A is required for efficient production of infectious HIV-1. PMID:22072966

<sup>11</sup> Cyclosporine blocks incorporation of HIV-1 envelope glycoprotein into virions. PMID:20181694

<sup>12</sup> Cyclosporine A-sensitive, Cyclophilin B-dependent endoplasmic reticulum-associated degradation. PMID:20927389

<sup>13</sup>  $\beta$ 2 Integrin Adhesion Complexes Maintain the Integrity of HIV-1 Assembly Compartments in Primary Macrophages. PMID:22017400

<sup>14</sup> Dynamics of HIV-1 Assembly and Release. PMID: 19893629

<sup>15</sup> A SNAP-tagged derivative of HIV-1--a versatile tool to study virus-cell interactions. PMID: 21799764

<sup>16</sup> Live-cell visualization of dynamics of HIV budding site interactions with an ESCRT component. PMID :1394086