Strategies for Inhibiting Function of HIV-1 Accessory Proteins: A Necessary Route to AIDS Therapy?

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Abstract: The Human Immunodeficiency Virus (HIV) genome encodes three major structural proteins common to all retroviruses (Gag, Pol and Env), two regulatory proteins (Tat and Rev) that are essential for viral replication, and four accessory proteins (Nef, Vif, Vpu, Vpr). While accessory proteins were initially reported to be unnecessary for viral growth, their importance as virulence factors is now being more and more appreciated: they can dramatically alter the course and severity of viral infection, replication and disease progression. None of the HIV accessory proteins display enzymatic activity: they rather act altering cellular pathways *via* multiple protein-protein interactions with a number of host cell factors.

All currently approved anti-HIV drugs target *pol* and *env* encoded proteins. Therefore, widening the molecular targets of HIV therapy by additionally targeting accessory proteins may expand treatment options, resulting in high impact effective new therapy.

In this review we present the state of the art of compounds that target HIV accessory proteins. Most of the research has focused on the inhibition of specific accessory proteins/host cell partner interactions. Promising compounds have been found within different classes of molecules: small natural and synthetic molecules, peptides and proteins, oligonucleotides, in particular those used as RNA interference (RNAi) tools.

With the assortment of compounds available, especially against Nef and Vif functions, the demonstration of the clinical efficacy of the new anti-HIV-1 drugs targeting accessory proteins is next challenge.

Keywords: HIV-1, accessory proteins, inhibitors, Nef, Vif, Vpu, Vpr.

AIDS: STATE OF THE ART

The human immunodeficiency virus (HIV) is the etiological agent of the acquired immunodeficiency syndrome (AIDS) in humans. HIV establishes a persistent infection in human hosts, with the depletion of CD4+ lymphocytes, the major target cells of viral infection *in vivo*, eventually resulting in defective cellular immunity [1]. AIDS was first reported in the United States in 1981 and has since become a major worldwide epidemic. The global number of people living with HIV continues to grow, as does the number of deaths due to AIDS. In 2007, an estimated 33 million people were living with HIV, 2.7 million became infected and 2 million died of HIV-related diseases (UNAIDS/WHO AIDS Epidemic Update: July 2008).

To date, the US Food and Drug Administration has approved 26 anti-HIV drugs, which fall into six broad categories: i) nucleoside and ii) non-nucleoside inhibitors of reverse transcriptase, iii) protease inhibitors and iv) fusion inhibitors. Very recently v) an entry inhibitor that antagonizes CCR5 coreceptor and vi) an HIV integrase strand transfer inhibitor have been approved [2, 3].

Used alone these drugs are effective in reducing viral replication. However, the antiviral effect is only temporary as HIV rapidly develops resistance to all known agents. To circumvent this problem, combination therapy (also called highly active antiretroviral therapy, or HAART) has proven very effective at both reducing virus load and suppressing the emergence of resistance in a number of patients. In the US, where HAART is widely available, the number of HIV-related deaths has declined [4]. Despite the success obtained with HAART, approximately 30-50% of patients ultimately fail resulting in the emergence of viral resistance. Viral resistance in turn is caused by the rapid turnover of HIV during the course of infection combined with a high viral mutation rate. Incomplete viral suppression is thought to provide an environment for drug resistant variants to emerge. Even when viral plasma levels have dropped below detectable levels (<50 copies/ml) as a consequence of HAART, low-level HIV replication continues [5, 6]. Moreover, it now appears that many individuals may not be able to take HAART indefinitely, due to serious long-term side effects. As many as 40-60% of patients who have received HAART for greater than one year have developed symptoms of Cushing's Syndrome, with hyperglycemia, hyperlipidemia, centripetal fat distribution, and peripheral muscle wasting [7].

Clearly there is a need for new antiviral agents, preferably targeting other viral components to reduce the rate of resistance and suppress viral replication even further.

The HIV-1 genome encodes three major structural proteins common to all retroviruses (Gag, Pol and Env), two regulatory proteins (Tat and Rev) that are essential for viral replication, and four accessory proteins (Nef, Vif, Vpu, Vpr) (Fig. (**1**)). Accessory proteins are multifunctional tools that the HIV virus (and simian immunodeficiency virus (SIV)) has developed to make host biology promote the virus life cycle. Studies *in vivo* or in primary cell types susceptible to HIV infection, have demonstrated that the accessory gene products can dramatically alter the course and severity of viral infection, replication and disease progression. Moreover, soluble HIV proteins exert bystander effects on neighboring immune cells in the absence of productive infection [8].

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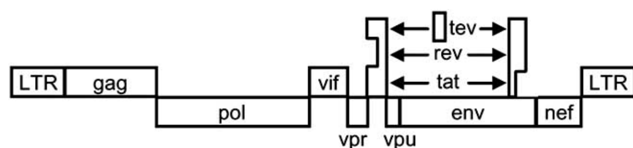

Fig. (1). Organization of the HIV-1 genome. Boxes indicate open reading frames. The Tev orf is isolate specific. The long terminal repeats (LTR) are present at both ends of the proviral DNA. "Structural Genes"**:** *gag* (Group-specific Antigen) codes for p24, the viral capsid; p6 and p7, the nucleocapsid proteins; and p17, a matrix protein; *pol* codes for viral enzymes, reverse transcriptase, integrase, and protease which cleaves the proteins derived from *gag* and *pol* into functional proteins; *env* (for "envelope") codes for the precursor to gp120 and gp41, proteins embedded in the viral envelope which enable the virus to attach to and fuse with target cells. "Transactivation Genes", genes essential for replication *in vitro:* Tat (**tr**ans-**a**ctivator of viral **t**ranscription) binds to **tat**-**r**esponsive RNA element (TAR), upregulates viral transcription; Rev (**re**gulator of **v**iral expression) binds to **r**ev-**re**sponsive RNA element (RRE), regulates viral RNA transport and splicing. "Accessory Genes", genes not essential for replication *in vitro*: Nef (**ne**gative **f**actor) downregulates CD4, MHC-I, binds cellular kinases, essential for viral disease induction *in vivo*; Vpu (**v**iral **p**rotein, **u**nknown) downregulates CD4, MHC-I, promotes virus release, not in HIV-2; Vif (**v**irion **i**nfectivity **f**actor) facilitates virion maturation; Vpr (**v**iral **p**rotein, **r**egulatory) arrests cell proliferation.

All currently approved drugs target HIV-1 Pol and Env encoded proteins (i.e. reverse transcriptase (RT), protease (PR), integrase (IN), gp41). Therefore, widening the molecular targets of HIV therapy by additionally targeting regulatory and accessory proteins may expand treatment options, resulting in high impact effective new therapy. Drug development against Tat and Rev can be found in recent reviews [9, 10]. Here we present the current state of the art of compounds that target HIV accessory proteins.

NEF PROTEIN

Nef was originally named "negative factor" because it was thought to inhibit viral replication [11]. Successively, Nef was instead found to be an essential factor for efficient viral replication and pathogenesis *in vivo*; it also facilitates virus replication and enhances virions infectivity *in vitro* [12, 13]. Long-term non progressors, who are HIV infected individuals that do not display the typical manifestations of AIDS but only minor CD4 counts after extended time, are commonly associated with either a deletion in the *nef* gene or defective *nef* alleles [14, 15]. In addition, rhesus macaques infected with an engineered strain of SIV that lacked the functional Nef protein also did not attained high virus loads and did not progress to clinical disease [16].

STRUCTURE

The HIV-1 Nef is a 25 KDa myristolylated protein. HIV-2 and SIV *nef* alleles have an additional C-terminal tail of 10–30 amino acids. Because of the large degree of solvent-expose surface area with several disordered regions, it has been difficult to obtain a precise three-dimensional structure of the full-length protein; however, HIV-1 Nef fragments that cover the whole polypeptide chain have been structurally characterized using NMR and X-ray crystallography [17]. Schematically, HIV-1 Nef possesses a genetically diverse and structurally flexible N-terminal arm of about 70 residues, followed by a well-conserved and folded core domain of around 120 residues (Fig. (**2**)). The core domain is the only part of Nef that adopts a stable tertiary fold. The high sequence conservation of the core domain in all *nef* alleles implies that this region has the same structure in HIV-2 and SIV Nef, as it has in HIV-1 Nef. Most of the motifs that are critical for binding to different cellular proteins are in the core domain and are exposed to their binding partners (Fig. (**2**)). Three proximal helices of the Nef core domain could theoretically form a cavity accessible to drugs [18].

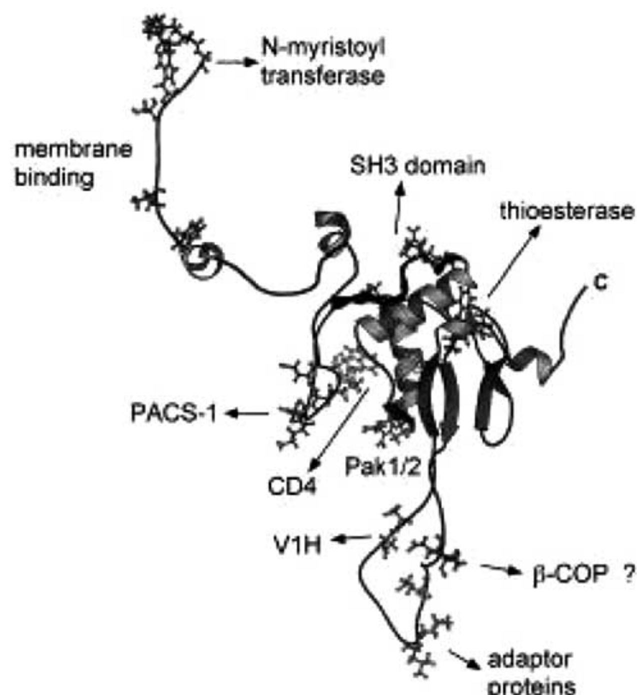

Fig. (2). HIV-1 Nef full-length structure as derived from the NMR structure assembly: functional motifs and their location. Binding sites for the known interacting proteins are indicated. Reprinted by permission from Macmillan Publishers Ltd: [EMBO Rep.] [17], copyright 2001.

The N-terminal anchor domain has been solved using NMR spectroscopy, and it appears that this domain adopts a relatively unstructured conformation that becomes partially ordered upon the addition of an N-terminal myristyl group [19]. It has been speculated that the overall flexibility of Nef enables the protein to switch between multiple conformations and that the structural organization of Nef may be dictated by its binding partner(s) [20].

Both the amino and carboxy termini are less conserved than the core domain and are thought to contain extended loop sequences. These sequences are enriched in short, linear signal sequences (i.e. tyrosine-based motifs, dileucine motifs, and diacidic motifs) that are known to be recognized by components of vesicular coats.

FUNCTIONS

HIV-1 and SIV Nef proteins have gained an important role in HIV pathogenesis, altering normal cell processes by several mechanisms (Table **1**). First of all it has been demonstrated that Nef promotes viral infection by activating CD4+ cytotoxic T lymphocytes (CTLs), rendering them suitable host cells [20-24]. Nef specifically alters signal transduction pathways downstream the T-cell receptor, affecting trasducers such as Vav [25], p21-activated kinase 2 [26], Rac, CDC42, and the DOCK2/ELMO1 complex [27]. In detail, Nef is able to interact with several tyrosine kinases of the Src family through its proline-rich (PxxP) motif that interacts with the kinase Src homology 3 (SH3) domains, and with several serine/threonine kinases [17, 28, 29]. During HIV or SIV infection, there is a widespread programmed cell death in infected and uninfected adjacent cells [22, 30]. Most of this apoptosis is mediated by Fas-Fas ligand (FasL) interactions: Nef-induced alteration of T-cell signalling pathway also leads to the upregulation of Fas ligand (FasL) on the cell surface, promoting apoptosis of neighbouring CTLs. On the other hand, to protect host cell from apoptosis Nef prevents signal transduction from FasL tumor necrosis factor (TNF) receptor and inhibits p53 and p21 mediated apoptosis [17]. Nef also protects infected cells by reducing cell surface expression of major histocompatibility complex class I (MHC-I), thus preventing viral antigen display on host cells avoiding CTL's activation [31]. Moreover, Nef selectively affects some MHC-I allotypes, and ignores others, thus avoiding lysis by natural killer (NK) cells, which recognize infected cells that lack sufficient surface MHC-I expression [17, 30, 32]. The most broadly investigated biological activity of Nef on host cells is the downregulation of CD4, HIV key cellular co-receptor. Although it may seem counterproductive that HIV-1 benefits from downregulating its own coreceptor, it is now clear that this function strongly correlates with improved viral pathogenesis. This is probably due to prevention of superinfection and enhancement of newly formed virion budding and release, leading to a controlled and productive infection [17, 33, 34]. In addition, Nef alters numerous intracellular trafficking pathways by binding to adaptor protein complex components, such as adaptor proteins (APs), coat protein 1 (COP-1) and phosphofurin acidic cluster sorting protein-1 (PACS-1) or members of the clathrin mediated endocitosis, such as Dynamin 2 [17, 31, 35].

Recently, it has been reported that the ancestral SIV *nef* gene product has a protective role, decelerating HIV progression to AIDS. It acts down-modulating T-cell receptor (TCR)-CD3, thus reducing T-cell activation and consequent cell death, besides protecting infected cells against killing by CTL's. This evidence draws back the initial "negative role" given to Nef, as in primate lentiviruses it contributes to the non-pathogenic phenotype [24, 36, 37].

Recent data on Nef protein are described in [18 and 38].

NEF INHIBITORS

Nef inhibition could potentially replicate the effect of harboring a *nef* deletion or a non-progressor Nef sequence, both of which are known to hinder clinical evolution to AIDS.

Small Molecules

Based on the evidence that various batzellidine and crambescidin alkaloids inhibit HIV related protein–protein interactions, such as HIV gp120–CD4, CD4–p56^{lck}, and HIV-1 cell-fusion [62, 63], Weiss group studied the inhibitory effects of a library containing the natural products ptilomycalin A, batzelladine F, and 46 batzelladine- and

Table 1. Known Functions of Nef

Biological Activities & Functions	Mechanism	Binding Partners	Reference
Promotion of viral infection	Activation of CD4+ CTL		[38]
Immune system escape	Reduction of MHC I expression on cell surface	Citoplasmic tail domain of MHC I, AP-1, PACS-1, ARF6, ARF1, PI3-kinase	[39-43]
Immune system escape and enhancement of infectivity	Reduction of CD4 molecules display on cell surface	V1H, AP-2, ARF-1, beta-COP, Lck	[38, 43-45]
Alteration of signal transduction pathways downstream T-cell receptor	Binding to molecules acting downstream T-cell receptor	Vav, p21-activated Kinase, Rac, CDC42, DOCK2/ELMO1 complex	[27, 46-52]
	Upregulation of FasL on cell surface		[53]
Inhibition of host apoptosis	Activity suppression of kinases trasducing signal from FasL and TNF receptor	ASK1	[54]
	Inhibition of p53 activity	p53	[55]
	Association with cellular kinases	p21-activated kinases, PI3-kinases	[56]
Impairment of intracellular trafficking pathways	Alteration of cytosolic vescicular trafficking, endocytosis and budding	Heterotetrameric clathrin-AP complex (AP-1, AP-2, AP-3, AP-4) and associated accessory proteins, VH1, PACS-1, COP-1	[42, 44, 45, 57, 58]
Enhancement of viral replication and infectivity	Downregulation of celluar factors	MHC II, CD8,CD28, CD80, CD86, CCR5, CXCR4, transferrin receptor, mannose receptor, TNF, thioesterase	[38, 41, 59-61]

crambescidin-based analogs on Nef binding towards three cellular ligands, i.e p53, actin and p56^{lck} [64]. All three cellular proteins bind to the unstructured N-terminus of Nef. By means of phage display-based and direct binding assays, their competition-binding data demonstrated that at compound concentrations of 5 μM, nine guanidines from this library inhibited the three Nef–ligand interactions at levels of >80%. Three compounds, (**1**), (**2**), and (**3**), (Fig. (**3**)) exhibited >94% inhibition of the three Nef–ligand interactions. All active compounds interacted primarily with Nef and not with the cellular partners.

Structure-activity analysis prompted the following considerations: 1) the bicyclic or tricyclic guanidine motif is essential for modulating Nef inhibition, as these guanidine units could form bidentate hydrogen bonds to an appropriate Nef functionality, possibly a carboxylate side chain; 2) however, two guanidine subunits decrease inhibitory activity; 3) the aliphatic side chains must be flexible to permit these molecules to assume various binding conformations.

Unfortunately, all active compounds resulted too cytotoxic to allow tissue culture-based assays; therefore no data on the compound potential to inhibit Nef function *in vivo* has been reported.

Subsequently, the quanidine-derived library was screened against a Nef library, called Nef allelome, containing 10^7 different variants of Nef [65]. The basic idea was that small molecules active against most Nef clinical variants would be less prone to promote drug resistance.

Alkaloid (**3**) (Fig. (**3**)), which was one of the simplest and most flexible compounds among the tested derivatives, resulted the most active against both wild-type and allelome Nef. This study indicates that compounds with well-placed, fewer flexible functional groups can better adapt to resistance-conferring mutations, and can hence offer the broadest spectrum of inhibition against Nef.

The structurally well defined Nef Src homology 3 (SH3) binding surface (Fig. (**2**)) is involved in the interaction of Nef with intracellular protein kinases, including several members of the Src tyrosine kinase family, and in particular with Hck, a Src family member expressed primarily in macrophages, an important HIV target cell type. Biochemical and structural studies have established that Nef binds specifically to the Hck SH3 domain with unusually high affinity. This interaction is bipartite in nature, involving a highly conserved Nef proline-rich motif (PQVPxR) and the surface of SH3, as well as a very specific interaction of the

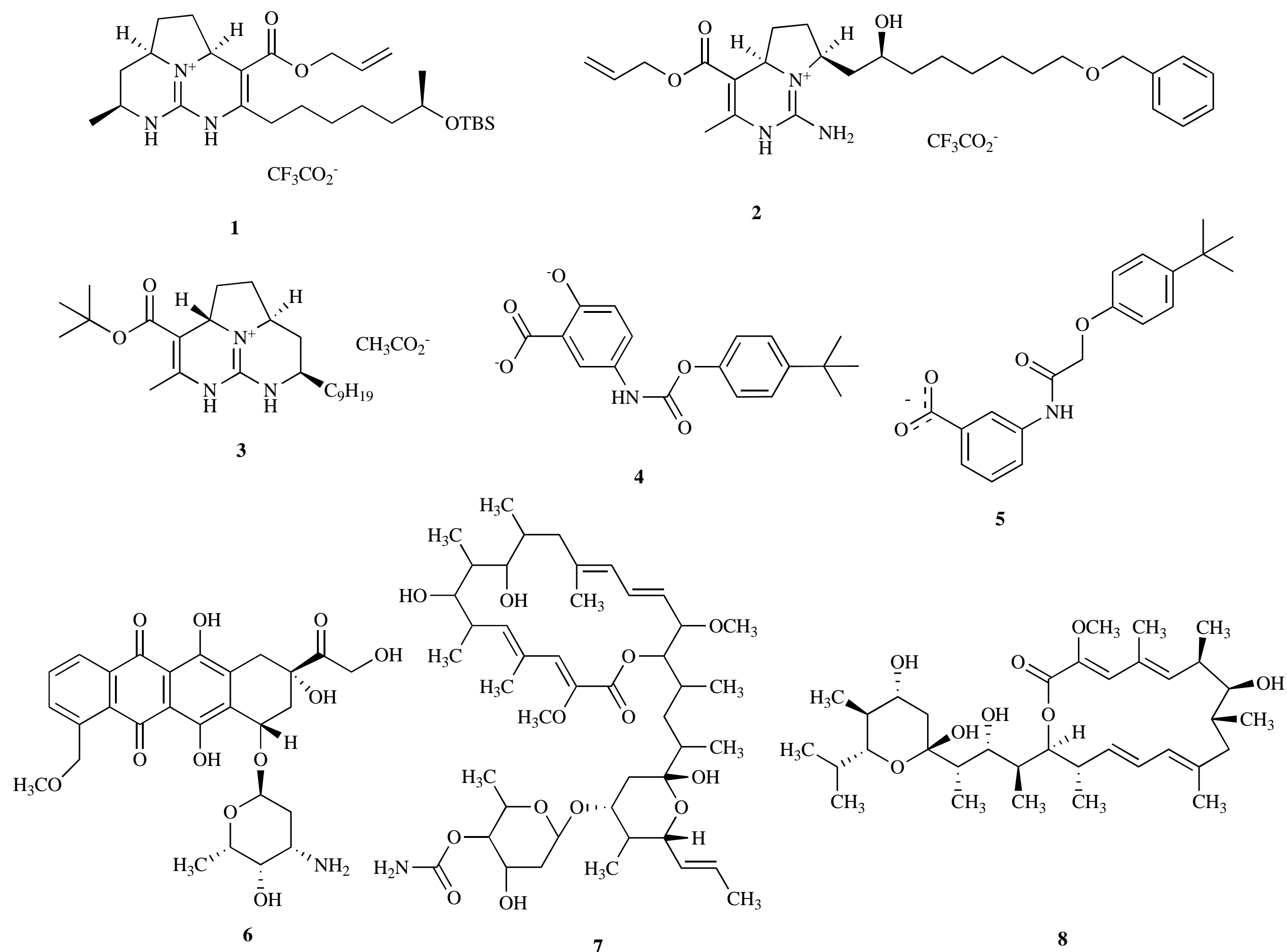

Fig. (3). Chemical structures of small molecule inhibitors of HIV-1 Nef function.

Hck SH3 domain RT loop and a conserved hydrophobic pocket of Nef [66]. Engagement of the Hck SH3 domain by Nef is sufficient to induce constitutive kinase activation both *in vitro* and *in vivo*. Given the well characterized binding of Nef with the SH3 domain of Hck and the biological relevance reported for this interaction [67], a number of studies focused on the discovery and development of specific inhibitors.

In a recent publication, Nef-Hck interaction inhibition has been effectively achieved by combining virtual and experimental screening [68]. High-throughput docking and application of a pharmacophoric filter on one hand and search for analogy on the other hand identified drug-like compounds that were further confirmed to bind Nef in the low micromolar range (K_d of 0.98 ± 0.3 μM, isothermal titration calorimetry), to target the Nef SH3 binding surface (NMR experiments), and to efficiently compete for Nef–SH3 interactions (cell-based assay, glutathione-S-transferase (GST) pull-down). Initial identification of these compounds by virtual screening was validated by screening of the very same library of compounds in the cell-based assay. The two best inhibitory compounds, (**4**) and (**5**), in both virtual and experimental screening are reported in Fig. (**3**) The two main chemical differences between (**4**) and (**5**) consist of (*i*) the transformation of the carbamate functionality into an amide group by a methylene insertion and (*ii*) the absence of phenolic function, leading to a benzoic acid bearing an *N*-acyl chain located in the *meta* position. The docking of (**4**) and (**5**) on Nef led to very similar models. In (**5**) the absence of a phenolic group allowing the formation of an intramolecular hydrogen bond with the carboxylic group of the benzoic acid, improves the possibility for additional electrostatic contribution with the Lys-82 from Nef. The theoretical calculation of the log *P* values predicted (**5**) to be a more soluble molecule as compared with (**4**), and neither (**4**) nor (**5**) produced significant cell toxicity in cell viability assays for concentrations up to 100 μM, which might be important for drug development.

All these studies targeting Nef SH3 binding surface, provide essential templates for future development of biologically active anti-Nef derivatives and new classes of antiviral molecules.

Another mammalian two-hybrid system to screen inhibitors of the interaction between Nef and Hck was developed by Murakami *et al.* [69]. By testing 500 samples from a library of natural and synthetic compounds they found that the four more effective samples (EC_{50} in the nanomolar range) were adriamycin derivatives (Fig. (**3**)). Adriamycin (**6**) is an anticancer drug that inhibits DNA topoisomerase by intercalating into the DNA. It was found that this drug inhibited Nef-Hck interaction by reducing the amount of Hck: hence, this activity was most likely related to the drug unspecific inhibition of DNA processing.

Finally, two macrolide antibiotics, inhibitors of vacuolar type H^+-ATPase, concanamycin B and bafilomycin A1 ((**7**) and (**8**), respectively, Fig. (**3**)) inhibited Nef-induced CD4 degradation in human cell lines at nanomolar concentrations [70]. Predictably, CD4 accumulated in the lysosomes and did not recover surface expression, indicating that the two antibiotics act through inhibition of acidification of lysosomes and do not affect cell membrane protein uptake and endocytosis.

mRNA, siRNA

Recently, research on Nef inhibitors has focused on RNA interference (RNAi) mechanisms. RNAi is triggered by double-stranded RNA (dsRNA) molecules, which are processed in the cytoplasm by the dsRNA-specific endonuclease Dicer into 19–24 nucleotides small interfering RNAs (siRNAs) or micro-RNAs (miRNAs). These si/miRNAs are incorporated into the multiprotein RNA-induced silencing complex (RISC) that guides the recognition and ultimately the cleavage or translational repression of complementary single-stranded RNA, such as messenger RNA or viral genomic RNA.

HIV is a rational target of RNAi because of its single-stranded RNA genome: unspliced genome-length and spliced subgenomic viral RNAs are possible targets for RNAi in the cytoplasm. RNAi against the *nef* gene, along with *tat* and *rev*, has been most widely studied because targeting early regulating viral genes may augment the efficacy of antiretroviral therapies. In addition, *nef* is localized at the 3' end of the HIV genome and it is hence contained in many viral transcripts. Suppression of HIV-1 replication has been achieved both in transiently and stably transfected cells by siRNA and short-hairpin RNA (shRNA) targeting *nef* or the portion of the *nef* gene overlapping the U3 region [71-76]. However, the rapid emergence of HIV escape mutants has severely limited the development of si/shRNA as therapeutic antiviral agents. In fact, a drawback of the high RNA sequence specificity is that single nucleotide substitutions or deletions or mutations that alter the local RNA secondary structure in the target region abolish inhibition [77]. The simultaneous use of multiple siRNA, or long-hairpin RNA (lhRNA) or long double-stranded (dsRNA) are some of the strategies applied to reduce the chance of viral escape by targeting the *nef* gene [78-80]. Some publications/patents presented miRNA-based approaches, which do not require perfect sequence complementarity [81, 82]. Other Nef targeted miRNAs were computationally designed within the *nef* gene of HIV-1 genome and further validated by experimental means. The validation was carried out in cell culture models. The miRNAs designed within the *nef* gene showed dependence on the miRNA since expression levels from the clone carrying the target region were downregulated compared to the expression from the vector without the target regions. Anti-miRNA could partially restore the reporter activity, while an unrelated anti-miRNA had no such effect proving that it was not a non-specific effect of DNA oligos [82].

Unfortunately, while all reports showed promising data on RNAi activity, none of them has lead to clinical trial applications.

Peptides

A third inhibitory Nef-directed approach involved the use of synthetic peptides that abrogated binding with a Nef cellular partner. In one report, Hck-derived SH3 domains showed potent inhibition of SH3-dependent Nef functions, such as

association with p21-activated kinase-2 and induction of the transcription factor NFAT, and did not compete with physiological SH3-mediated processes [83].

More recently, an artificial 12 amino acid proline-rich peptide (PD1: acetyl-HSKYPLPPLPSL-amide)) obtained from a phage display screening against the SH3 domain, exhibited exceptional affinity for the Hck SH3 domain (K_d = 0.23 μM) and was able to competitively displace Nef from Hck SH3 by competition for the PXXP binding site [84].

VPU PROTEIN

In 1988 a new unknown (U) virus protein (VP) was identified in the HIV-1 genome. This protein, called Vpu, is found only in HIV-1 and SIVs isolated from chimpanzees (SIVcpz), and three species of old world monkeys within the genus Cercopithecus (SIVgsn) [85]. HIV-1 Vpu is highly conserved: strains from several isolates show a high level of sequence identity and homology over the entire sequence, making the protein a potential drug target [86]. There is evidence of a role of Vpu in HIV-1 pathogenesis. In animals infected with viruses where *vpu* deletions are sufficiently large to prevent reversions, long-term non-progressing infections characterized by lack of CD4+ T cell depletion were observed [87].

STRUCTURE

Vpu is an 81-aminoacid long protein with a molecular weight of 9 KDa. It was identified as a type I integral membrane protein. Residues 1-27 constitute the N-terminal hydrophobic membrane anchor. The remaining 54 residues protrude into the cytoplasm (Fig. (**4**)). This part includes 15 charged aminoacids: a membrane proximal stretch of basic residues followed by acidic residues that confer an overall negative electrostatic charge to the molecule. This cytoplasmatic part contains two phosphorylation sites at two serine residues (Ser-52 and Ser-56). The membrane-spanning N-terminal domain forms a stable α-helix connected to the soluble cytoplasmatic tail by a short unstructured fragment. The cytoplasmatic domain is formed by two discrete α-helical structures encompassing aminoacid positions 35-50 and 58-70, separated by a flexible segment containing the two conserved phosphorylated serine residues. However, the Vpu tertiary structure is probably affected by its interaction with cellular factors, which it has been shown to interact with.

In addition, the Vpu protein is capable of homo-oligomerizazion into higher molecular weight structures of up to 80 KDa in the presence of membranes and 85 KDa in the absence of membranes. Based on these values, it was suggested at least a tetramer as a putative oligomerization state *in vivo*, which may significantly impact the overall Vpu conformation in membranes.

FUNCTIONS

Vpu primary function is to induce degradation of CD4 molecules in the endoplasmatic reticulum (ER). Both Nef and Vpu viral proteins downregulate CD4 in host cells. Nef and Vpu are mainly expressed early and late, respectively, in the viral life cycle, ensuring continuous removal of CD4. Nef (see above) links mature CD4 to components of clathrin-dependent trafficking pathways at the plasma membrane, and perhaps in intracellular compartments, leading to internalization and delivery of CD4 to lysosomes for degradation. Vpu, on the other hand, interacts with newly-synthesized CD4 in the endoplasmic reticulum, linking CD4 to the Skp1–Cul1–F-box-protein (SCF) ubiquitin ligase and facilitating the entry of CD4 into the endoplasmic-reticulum-associated degradation pathway. CD4 downregulation is additionally performed by the gp160 envelope glycoprotein precursor (Env). However, CD4-gp160 complexes remain trapped in the ER, thus blocking transport and maturation of both CD4 and the Env protein itself [88]. Vpu induces degradation of CD4 molecules trapped in complexes with Env, thus allowing gp160 to resume transport toward the cell surface. Vpu physical interaction with CD4 in the ER targets CD4 to the degradation pathway [89]. The determinants for CD4 degradation are all contained in the cytoplasmatic domain of Vpu: the viral protein regions responsible for this interaction are residues 28-47 and 76-81 at the C-terminal [90]. The Vpu phosphorylated cytoplasmatic residues are crucial for directing the Vpu-CD4 complex to the ubiquitin-dependent proteosome degradation pathway, but they are not strictly required for the protein binding to CD4.

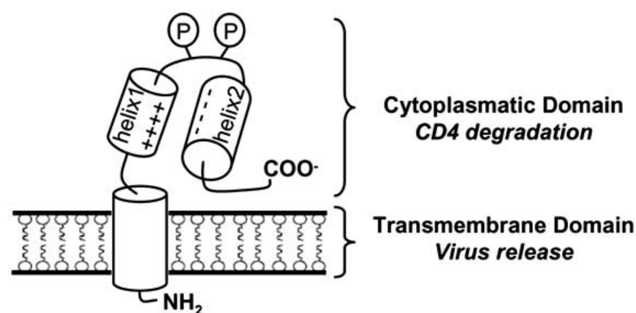

Fig. (4). Structural domains of Vpu. Vpu consists of an N-terminal hydrophobic domain, that functions as membrane anchor, and a hydrophilic cytoplasmic domain. The cytoplasmic domain contains two amphipathic *a*-helical domains of opposite polarity. They are separated by an unstructured region containing two conserved seryl residues which are phosphorylated by protein kinase CK-2. The cytoplasmic domain contains sequences critical for CD4 degradation while the membrane anchor domain has a critical function in regulating virus release and plays an important role in the formation of cation selective ion channels. Vpu forms homo-oligomeric complexes. Only the monomeric form is shown. A putative interaction between helix 1 and helix-2 as shown in the cartoon suggests only one of many possible conformations of Vpu [208]. Reprinted from Mol. Cell, 14, Strebel K., HIV-1 Vpu: putting a channel to the TASK, 3, Copyright (2004), with permission from Elsevier.

Vpu is also involved in the efficient release of viral particles from HIV-1 infected cells. This role is allocated to the transmembrane domain of Vpu. Though controversially, this activity has been linked to the Vpu ability to form ion channels [91], which appear to be selective for monovalent cations, such as sodium and potassium. It has been proposed that Vpu pore formation could also induce cellular factors

involved in the late stages of virus formation or exclude cellular factors inhibitory to the viral budding process [92]. Finally, it has been suggested that Vpu mediates the efficient membrane association of other proteins with the consequence of enhancing viral particle release [93].

Complete Vpu description is found in [86, 92, 94, 95].

VPU INHIBITORS

Small Molecules

Amiloride derivatives were the first small molecules structures reported to block Vpu function. In particular, 5-(N,N-hexamethylene)amiloride (compound (**9**)) and, to a lesser extent, 5-(N,N-dimethyl)amiloride (compound (**10**)) in Fig. (**5**) inhibited the budding of virus-like particles from HeLa cells when they were expressing Gag and Vpu proteins from HIV-1 [96]. Experiments with both full-length Vpu and with a peptide corresponding to its N-terminal region, reconstituted into lipid bilayers, indicated that the derivatives block channel activity, thus linking the blocking to Vpu. Compound (**9**) has also been reported to inhibit another viral channel protein, p7 from hepatitis C virus [97] and amiloride itself is known to block the epithelial Na^+ channel [98]. However, experimental binding constants have not yet been measured.

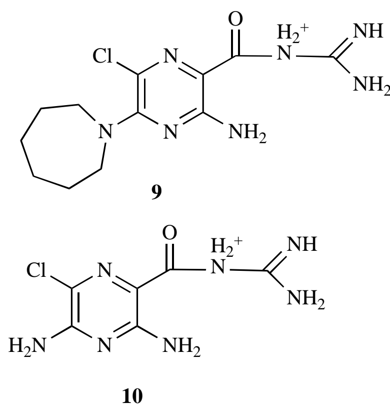

Fig. (5). Chemical structures of small molecule inhibitors of HIV-1Vpu.

Computational studies established that compound (**9**) forms hydrogen bonds with Ser-24 and tryptophans located at the helix/lipid interface. Concurrently, compound (**9**) hydrophobic ring was shown to be involved in hydrophobic interactions with Vpu hydrophobic residues towards the N-terminal end [99]. Based on these models, it was suggested that the binding site of the blocker (**9**) lies within the channel. The mode of compound (**9**) inhibition, once it is in the pore, is that of steric hindrance. This steric hindrance is supported by a combination of factors such as hydrogen bond formation with tryptophan and implementation of slightly altered pore geometry. In contrast, compound (**10**), although binding within the pore, is still able to allow for space for water molecules and ions to pass through [100].

Therefore, rational structures of inhibitory Vpu ligands should contain both hydrogen acceptor groups and a spacious hydrophobic tail to block the pore.

Very recently, amphotericin B methyl ester (AME), a cholesterol-binding compound, was reported to inhibit HIV-1 particle production [101]. Initial experiments indicated that the mechanism of inhibition by AME is distinct from that by cholesterol depletion, since AME had no significant effects on Gag binding to the plasma membrane, Gag association with lipid rafts, or Gag multimerization. AME significantly disrupted virion morphology and its activity was related to the presence of Vpu, since AME did not inhibit the release of Vpu-defective HIV-1 or Vpu-minus retroviruses, such as murine leukemia virus (MuLV) and SIV. Interestingly, AME markedly reduced the ability of Vpu to counter the activity of CD317, also called BST-2, HM1.24 or tetherin (see below); hence AME is the first small molecule reported to interfere with the anti-CD317 function of Vpu.

Proteins

Last discovery in terms of Vpu inhibitors is called CD317 [102]. Neil and colleagues initially demonstrated that an inhibitory factor(s) for virion release was induced by interferon α (IFN-α) [103]. By means of a microarray analyses of mRNA expressed in human cell lines treated with or without IFN-α, they identified CD317 (also called BST2 or HM1.24) a membrane protein of previously unknown function, which was called “tetherin”.

CD137 can be constitutively expressed or induced by interferon-α and causes retention of fully formed HIV-1 virions on infected cell surfaces. It was shown that CD317 expression correlated with a requirement for Vpu during HIV-1 particle release. Furthermore, in cells where HIV-1 virion release required Vpu expression, depletion of CD317 abolished this requirement. CD317 caused retention of virions on cell surfaces and, after endocytosis, in CD317-positive compartments. Vpu co-localized with CD317 through the transmembrane (TM) domain of Vpu in intracellular compartments and inhibited these effects.

CD317 is an integral membrane protein that has an N-terminal cytoplasmic tail followed by a TM domain, extracellular coiled-coil domain and a glycosyl phosphatidylinositol (GPI) anchor at the C-terminus. CD317 localizes at the cell surface, probably in lipid rafts. The GPI anchor localizes within rafts but the TM domain resides outside the rafts. CD317 also resides in an intracellular pool and cycles between the intracellular pool and the cell surface. During this cycle, clathrin adaptor AP-2 interacts with the cytoplasmic tail of CD317 and triggers clathrin-mediated endocytosis [104].

Even though some hypothesis have been made [95], the molecular basis for tetherin’s antiviral activity and for counteraction by Vpu have not been precisely elucidated. Nonetheless it is clear that inhibition of Vpu function and consequent mobilization of tetherin’s antiviral activity is a potential therapeutic key strategy in HIV/AIDS.

VPR/VPX PROTEIN

The *vpr* gene was isolated in all HIV and SIV genomes, while a *vpx* gene in addition to *vpr* has been found in HIV-2 and SIV from other lineages, i.e. SIVmac (rhesus), SIVsm

(sooty mangabey), SIVmnd-2 (mandrill), SIVrcm (red-capped mangabey) and SIVdrl (drill) [105]. The Vpr and Vpx proteins share considerable sequence similarity and are thought to have arisen by duplication of a single ancestral gene; they both encode small proteins of approximately 100 amino acids.

The viral protein R (Vpr) of HIV-1 is a small basic protein of 96 amino acids (14 kDa). The importance of Vpr in promoting HIV-1 pathogenesis has been proved by several observations: mutations to key amino acids have been associated with long-term non-progressive HIV infections [106], and monkeys infected with a SIV that did not express the *vpr* and *vpx* genes displayed a very low virus burden and did not developed the immunodeficiency disease [107]. However, Vpr/Vpx functions during the natural course of infection have not been yet clarified.

STRUCTURE

Although the full-length Vpr protein aggregates in solution, the tertiary structure of the protein has been assessed by NMR analysis of a soluble full-length Vpr (1-96) polypeptide [108]: the protein is constituted by three amphipathic α-helices (D17-H33, W38-Y50, T55-R77) (Fig. (**6**)).

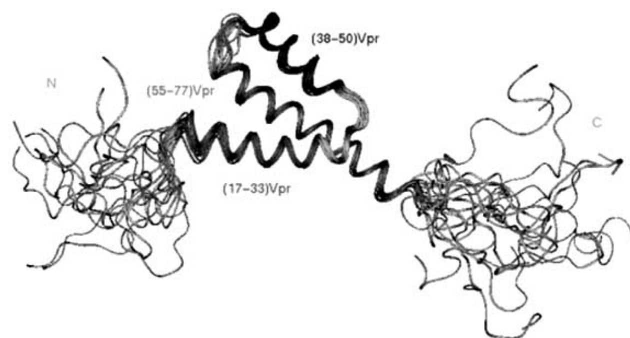

Fig. (6). NMR structure of the HIV-1 regulatory protein Vpr. Backbone superimposition of 15 selected structures of (1–96) Vpr, performed on the (17–33), (38–50) and (54–77) α-helices [108]. Reprinted from J. Mol. Biol., 327, Morellet N., Bouaziz S., Petitjean P., Roques B.P., NMR structure of the HIV-1 regulatory protein Vpr, 13, Copyright (2003), with permission from Elsevier.

The helix encompassing residues 55-78 is rich in leucine residues, which, along with hydrophobic residues on one side of the helix, can form a leucine zipper motif. This structure may account for the formation of Vpr dimers. The three helices are connected by loops and are folded around a hydrophobic core surrounded by a flexible N-terminal domain and a C-terminal arginine-rich region that are negatively and positively charged, respectively. The N-terminal domain contains four conserved prolines which are important for the correct folding of the viral protein; the C-terminal region displays six arginines which may be responsible for some Vpr functions, such as its transducing properties and ability to cross the cell membranes.

FUNCTIONS

Vpr is expressed at a late stage of the virus life cycle, but it is present during the early steps of infection of target cells since it is packaged into virions released from the producing cells. Vpr has a potential role in the initiation step of the reverse transcription process since it has been shown that it is present in the reverse transcription complex along with the two copies of viral RNA and other viral proteins, such as RT, IN and NCp7 [109, 110]. In addition, Vpr modulates the *in vivo* mutation rate of HIV-1 by influencing the accuracy of the reverse transcription, which is four time less faithful in the absence of Vpr expression. This activity derives from the interaction of Vpr with the nuclear form of uracil DNA glycosylase (UNG2), an enzyme involved in the base excision repair pathway that specifically removes the RNA base uracil from DNA [111]. Despite the lack of any identifiable canonical nuclear localization signal (NLS), Vpr is rapidly targeted to the host cell nucleus after infection and it is then able to shuttle between the nucleus and the cytoplasmatic compartments. Vpr is an integral component of the pre-integration complex (PIC), where the viral DNA associates with IN and other viral and cellular proteins. It has been proposed that Vpr enhances the transport of the viral DNA into the nucleus by promoting direct or indirect interactions with the cellular machinery regulating the nucleo-cytoplasmatic shuttling [112]. Vpr molecules packaged into infected virions induce an arrest in the G2 phase of the cell cycle of infected T cells and there is evidence that the cellular pathway altered by Vpr is well conserved in all eukaryotic cells. Since HIV-1 long terminal repeat (LRT) seems to be more active in the G2 phase, it is possible that Vpr confers a favorable cellular environment for efficient transcription of HIV-1 by arresting the cell cycle at G2. Vpr represents one of the multiple pathways through which HIV induces apoptosis, and eventually depletion of $CD4^{+}$T cells. Vpr induction of apoptosis is mediated by permeabilization of the mitochondrial membrane and subsequent dissipation of the mitochondrial transmembrane potential [113]. Different evidence suggests that this effect is generated by either a direct action of Vpr on mitochondria [114], or by the physical interaction of Vpr with the adenine nucleotide translocator (ANT) [115], which triggers caspase-mediated apoptosis [116]. In addition, extracellular Vpr also displayed apoptotic activity: it was proposed that Vpr found in sera and cerebrospinal fluid of HIV-1 infected patients, may play a role in the development of AIDS-associated dementia [117]. Vpr increases the transcriptional activity of both the viral LTR and cellular promoters. This effect is mediated by the interaction with transcription factors: in fact, Vpr displays DNA sequence specificity only when complexed with cellular partners [118]. Hence, Vpr may function as an adaptor molecule for efficient recruitment of transcriptional co-activators. Finally, it has been recently reported that Vpr is required for efficient Nef expression from unintegrated viral DNA, linking the activity of Vpr to that of Nef [119].

For a complete recent review on Vpr and its functions see [120].

VPR/VPX INHIBITORS

Small Molecules

Recently two natural compounds have been shown to inhibit Vpr function. Using a screening system of fungal me-

tabolites in budding yeast cells expressing Vpr, Watanabe *et al.* identified fumagillin as antagonizer of Vpr-mediated growth inhibition in yeast cells [121]. Fumagillin (compound (**11**), Fig. (**7**)) is a compound already known to be a potent inhibitor of angiogenesis; in addition, it is already successfully used in the clinic to treat Kaposi's sarcoma and microsporidiosis in AIDS patients. A possible mechanism of action for fumagillin and its synthetic derivative TNP470 (compound (**12**), Fig. (**7**)) was reported to be the inhibition of Vpr-mediated G_2 arrest in mammalian cells. However, it is still unknown if the effect of fumagillin on Vpr is exerted by direct binding or is mediated by downstream pathways. Affinity binding assays failed to prove direct binding, but the affinity might have been too weak to be detected in such assay.

By screening small molecule libraries including ion channel inhibitors, kinase/phosphatase inhibitors, orphan ligands, endocannabinoids and bioactive lipids, damnacanthal stood up as the best inhibitor of Vpr-induced cell growth cessation in mammalian cells [122]. Damnacanthal is an anthraquinone derivative first isolated from noni fruit (compound (**13**), Fig. (**7**)), a traditional Tahaitian fruit commonly used as a folk medicine by Polynesians for over 2000 years. Contrary to fumagillin, damnacanthal inhibits Vpr-dependent apoptosis without affecting the induction of G_2 arrest or maintenance [123]. Hence, it was proposed that damnacanthal can help distinguishing Vpr-induced cell death mechanisms.

Based on the observation that the cell cycle arrest induced by Vpr is similar to that observed in cells arrested in G_2 by treatment with DNA-damaging agents such as the classical bifunctional alkylating mutagen nitrogen mustard (mustine*,* HN2), Poon *et al.* reported that pentoxifylline (compound (**14**), Fig. (**7**)) is active in reducing Vpr-mediated cell cycle arrest at concentration of 0.5 to 1.0 mM [124]. Pentoxifylline is a member of the methylxanthine family which has been used in a number of clinical settings [125, 126] and treatment with methylxanthines in conjunction with alkylating agents has been shown to abrogate the G2 delay. Pentoxifylline did not affect Vpr expression or localization, but more studies would be needed to elucidate its mechanism of action. In fact, pentoxifylline has been recently suggested to inhibit HIV-1 transduction and replication at least partly by suppression of the ATR kinase [127], indicating the need for more detailed studies to prove the specificity of pentoxifylline effect on Vpr. *In vivo* administration of pentoxifylline did not show decrease in the viral load of AIDS patients, however, the concentration of pentoxifylline achievable in patients (5 to 10 μM) would have been insufficient to have an effect on Vpr, given the much higher levels required *in vitro* (0.5 to 1 mM) to alleviate the cell cycle arrest. Hopefully, the effects of pentoxifylline as lead compound may assist in the identification of other more potent derivatives which are effective at concentrations achievable *in vivo*.

Oligodeoxynucleotides

Among a series of unmodified phosphodiesters oligonucleotides (PO-ODNs) complementary to some of the HIV-1 regulatory genes, PO-ODN sequences complementary to the *vpr* gene emerged as potent inhibitors of HIV-1 multiplication in *de novo* infected cells (EC_{50} 0.8-3.3 μM) [128]. These PO-ODNs were not cytotoxic *per se* and were not inhibitory to HIV-1 multiplication in chronically infected cells. Structure-activity relationship studies indicated that their antiviral activity was not related to an antisense mechanism, but to the presence, within the active sequences, of contiguous guanine residues. These guanine clusters could spontaneously fold in G quartet/quadruplex structures: this conformation on one hand rendered the PO-ODNs stable to degradation, and, on the other hand, conferred properties to specifically interfere with events on the HIV-1 life cycle that followed the adsorption step and preceded the reverse transcription.

Peptides

Two different genetic selections permitted to identify Vpr binding peptides with inhibitory activity. A random hexameric peptide library fused to the C-terminus of a GST inert carrier protein was screened in *S. cerevisiae* budding yeast.

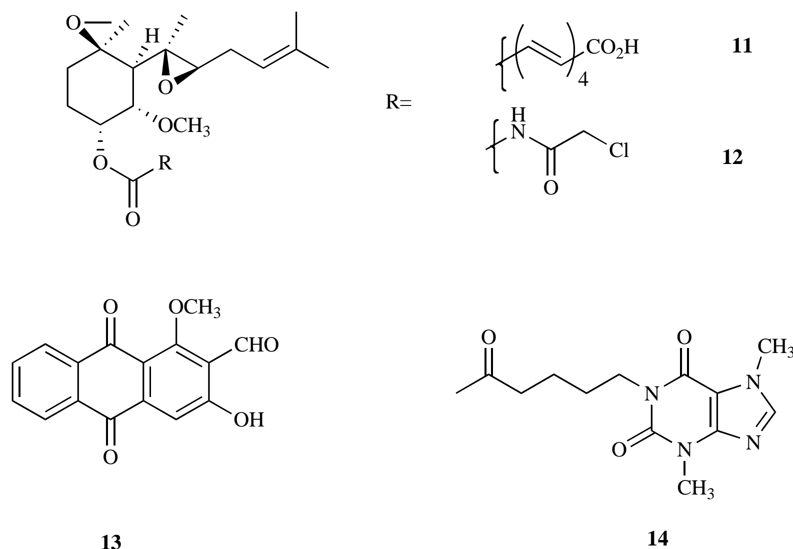

Fig. (7). Chemical structures of small molecules inhibitor of HIV-1 Vpr.

The genetic selection identified 15 peptides that suppressed the growth arrest phenotype of HIV-1 Vpr [129]. This effect correlated with the peptide binding affinity for Vpr, as detected by GST pull-down binding assay. All the 15 peptides contained a conserved di-W motif which was suggested to be critical for the peptide ability to interact with Vpr and for inhibition of its biological activity. In addition, the most potent peptides displayed a stretch of hydrophobic residues at their C terminus that could be responsible for part of the inhibitory activity. Four peptides harbored a WXXF motif, which is present in uracil DNA glycosylase (UDG) that specifically binds to Vpr to increase reverse transcriptase fidelity. The selected peptides were shown to alleviate Vpr-mediated apoptosis and cell cycle G2 arrest, to colocalize with Vpr in mammalian cells and to interfere with the protein nuclear translocation.

A random heptapeptide phage-display library was used to select Vpr binding peptides [130]. The identified peptides were further confirmed by using the yeast two-hybrid system for intracellular interactions of Vpr with peptides. The great majority of these peptides contained the consensus motif WXXF, which is shared with UDG.

The described two different genetic selection methods identified a common motif which bind and inhibit some of Vpr function and could thus be exploited for development of more potent inhibitors.

By using a peptide library that spans the 96-residue-long Vpr protein, it has been recently shown that some Vpr-derived peptides can bind both HIV-1 RT and IN [131]. In particular, peptides derived from the C-terminal domain of Vpr were the best binders of conserved regions of both RT and IN. The vast majority of the binding peptides also decreased target protein activity. This property was proposed to be the result of steric hindrance or conformation changes in the RT and IN active sites, whereas a blockage of the dimerization state was attributed to the inhibition of IN. Since therapeutic properties were shown for peptides targeting HIV-1 RT and IN that inhibited viral replication in HIV-1 infected cells, such characteristics must be pursued in the design and development of novel and potent Vpr-derived peptides.

Dominant Negative Mutants

Alterations of amino acids within the Vpr leucine-rich domain at position 73 from arginine to serine (R73S), rendered Vpr defective in stimulating transcription and replication of the HIV-1 genome in the presence of wild-type Vpr and the viral transactivator, Tat [132]. Production of the mutant Vpr interfered with the replication of the wild-type and delta Vpr virus in the cells. Accordingly, a Vpr mutant virus containing the transition of arginine to serine at position 73 exhibited an inhibitory effect on the replication of wild-type virus. In addition, the Vpr mutant 73 (Arg to Ser), which displayed dominant negative properties, retained its stability and ability to incorporate into virions and to localize in the nuclei of the infected cells.

Vpr was reported to enhance the activity of glucocorticoids in mammalian cells by interacting directly with the glucocorticoid receptor and general transcription factors, acting as a coactivator. In fact, Vpr contains the signature motif LXXLL also present in cellular nuclear receptor coactivators, such as steroid receptor coactivator 1, which mediates their interaction with the glucocorticoid and other nuclear hormone receptors. It was shown that a mutant Vpr molecule with disruption of this coactivator signature motif (RU486) lost its ability to influence transcription of glucocorticoid-responsive genes and became a dominant-negative inhibitor of Vpr, possibly by retaining its general transcription factor–binding activities [133]. Mutations in the LXXLL region also decrease the nuclear localization of Vpr.

These novel Vpr dominant negative mutants may provide an effective strategy for the development of HIV-1 targeted therapeutics.

Vpr as Vector

Besides being a target for antiviral therapy, the Vpr protein is an attractive molecule for the development of antiviral agents targeting the events associated with virus maturation because of its specificity for the HIV virus particle. Based on this principle, Vpr has been fused to anti-integrase single-chain variable antibody fragments [134], to sequences corresponding to nine cleavage sites of the Gag and Gag-Pol precursors of HIV-1 [135], and to enzymatically active or inactive viral proteases [136, 137]. All these approaches proved to be successful in decreasing the viral replication and infectivity of virions into which the chimeric protein was encapsidated.

Hence Vpr could be effectively exploited not only as antiviral target, but also as antiviral agent delivery vector.

VIF PROTEIN

The Virion Infectivity Factor (Vif) is a 23 KDa, highly basic phosphoprotein that is present in all lentivirus with the exception of equine infectious anemia virus (EIAV). It is produced late in the infection cycle, in a Rev-dependent manner. Vif was reported to be essential for efficient viral replication in a cell-dependent manner: deletions in the *vif* gene have been associated with a reduction or loss of viral infectivity in non-premessive cells, while permissive cells do not require a functional *vif* gene product.

STRUCTURE

Although the high conservation of the open reading frame implies an important role of Vif, its amino acid sequence is not highly conserved between different viruses pointing to specialized virus- and species-specific functions [138]. A detailed structural analysis of Vif has been so far precluded by non-specific aggregates that form during purification and enrichment [139]. Hence, all data on functional domains of Vif are based on mutational analysis. The Vif sequence has no predicted functional domain structure: residues shown to be important for activity are distributed throughout the protein sequence (Fig. (**8**)).

The N-terminus is necessary for RNA binding [140] and a stretch of basic amino acids located near the C-terminus of Vif confers membrane association properties [141]. The C-

terminal 22 amino acids have been also involved in the interaction of Vif with Gag [142]. Other important sequences include a SLQ well conserved motif in the 114-148 central region, and a HCCH motif; multiple phosphorylation sites (Thr96, Ser144, Thr155, Ser165, and Thr188), two conserved cysteines at positions 114 and 133 and a PPLP motif (amino acids 161-164) [143]. All these residues are required for Vif activity. Finally, a proline-rich domain encompassed by residues 151-164 was found to be important for Vif multimerization [144].

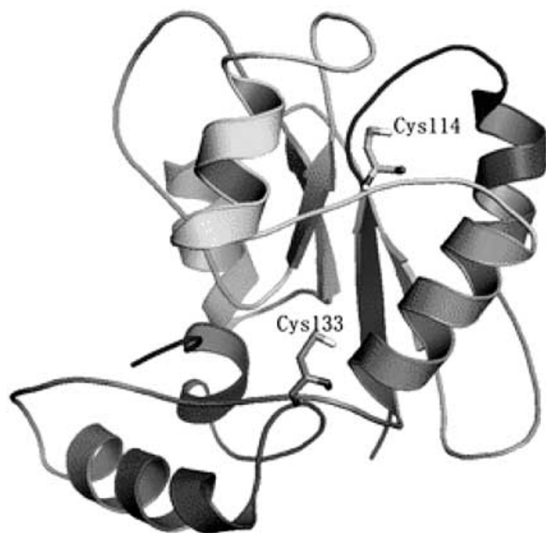

Fig. (8). Three-dimensional structure of HIV-1 Vif constructed by comparative molecular modelling [209]. Reproduced by permission of The Royal Society of Chemistry.

FUNCTIONS

The best well-characterized function of Vif is its ability to neutralize ABOBEC3G. APOBEC3G, initially designated as CEM-15 [145], is part of a large family of related enzymes with cytidine deaminase activity [146]. The normal cellular roles for APOBEC3G are unknown, but evidence suggests that it may be involved in a general host cell antiviral protective mechanism, not just restricted to HIV [147]. When APOBEC3G is present in viral producer cells infected with Vif-deficient HIV, it can introduce G to A mutations into reverse transcriptase products in newly infected cells [148, 149]. Even if the exact mechanism of Vif function is still under investigation, there is evidence that the inhibition of APOBEC3G antiviral activity is mediated by a physical interaction with Vif, and results in the exclusion of APOBEC3G from virions. A single mutation in the N-terminal part of APOBEC3G (D128K) is sufficient to change its sensitivity to Vif [150-153]. However, it is likely that other residues are important for the interaction with Vif, possibly through an RNA bridge [154]. Vif binding to APOBEC3G induces APOBEC3G protein degradation and prevents its incorporation into virions [155, 156]. Vif-stimulated APOBEC3G ubiquitination and proteasome-mediated degradation depends on Vif binding to components of a cullin-ubiquitin ligase complex, including Cullin5, Elongin B, Elongin C, and the RING protein Rbx [157-159]. The conserved motifs SLQ and HCCH mediate Vif interaction with the ubiquitin-mediated proteasome complex. However, there is increasing evidence that Vif prevents packaging of APOBEC3G through multiple mechanisms [154].

Vif incorporation into virions is non-selective and Vif packaged amounts are low and vary depending on the intracellular expression levels of Vif [160, 161]. It was found the packaged Vif stably associates with the viral genomic RNA [140]: consistently, Vif copurifies with viral reverse transcriptase, integrase and unprocessed Gag [154]. It is thus likely that, despite its low abundance, Vif exerts other critical functions during the early phase of viral infection as part of the HIV nucleoprotein complex.

In addition, Vif has been reported to interact with the cellular kinase Hck [162], with the zinc finger protein inhibiting NF-kB (ZIN) [163, 164], which inhibits TNF-α and IL-1 induced NF-kB mediated signaling [165], the spermidine/spermine N-actyl transferase (SSAT) [163], which is a key regulatory enzyme of cellular metabolism and catalyzes a rate limiting step in polyamine catabolism [166, 167], and SP140, a component of a subset of promyelocytic leukemia (PML) bodies or nuclear bodies/domains (ND) [168] that are associated with regulation of gene transcription, DNA replication and repair and the cell cycle [169].

These newly identified interactions of Vif with cellular partners might be important for the search and development of antiviral factors.

For recent complete reviews on Vif see [154 and 173].

VIF INHIBITORS

Many recent reviews indicated Vif as a new potential important target for anti-HIV therapy [170-172].

Possible ways to prevent Vif activity have been described by Carr *et al.* [173]). Most of them include disruption of Vif-mediated inhibition of the natural antiviral factor APOBEC3G. This would be achieved by acting on APOBEC3G by increasing its synthesis or its Gag/RNA binding or by decreasing its degradation; by acting on Vif by decreasing its synthesis, increasing its proteolysis or altering its cellular localization. In addition, inhibition of Vif functions maybe envisaged by interfering with interaction of Vif and other identified or yet to be identified cellular partners.

Here we describe the strategies applied and results so far achieved in experimental settings to reduce Vif-associated HIV infectivity.

Peptides and Proteins

Randomly Selected Peptides

Most of the experiments performed to find Vif inhibitors involved proteins and peptides more or less related to the Vif sequence.

A phage display assay was employed to identify a set of 12-mer peptides, all containing a PXP motif [174]. Among these proline-enriched peptides, the peptides containing the PXPXP motif had the higher binding affinity to Vif protein. Since it had previously been demonstrated that the ^{151}AALIKPKQIKPPLP^{164} domain of HIV-1 Vif is critical for

Vif multimerization, which is required for Vif function, it was proposed that all the identified peptides could inhibit Vif multimerization. Indeed, it was demonstrated that the ^{161}PPLP^{164} domain plays a key role in Vif-Vif interaction and that either random peptides containing the PXP motif, or Vif-derived peptides presenting the PPLP sequence, could effectively inhibit Vif-Vif interaction. In addition, the selected peptides were able to inhibit Vif-Hck binding and they could effectively enter HIV-1 infected cells as antennapedia homeodomain fusion peptides. They were also reported to inhibit HIV-1 replication in cell culture, although no EC_{50} value was reported. These data indicated that Vif multimerization is important for the function of Vif and presented a chemical structure which could be exploited for the design of more effective synthetic Vif inhibitors. Based on the results collected on their work, Zhang *et al*. issued two patents [175, 176] where the amino acid sequence from amino acid residue 144-177, 151-164, 161-164 were patented with many modifications, including peptidomimetics, non-classic amino acids, or chemical amino acid analogs and enzymatic or chemical modifications.

A precedent phage-biopanning method against Pr55Gag and Vif interacting domains had identified both Gag and Vif competitor peptides. The Gag competitors spanned a continuous region of Gag between His421 and Thr470. In the Vif competition experiments, fifty individual clones were isolated and sequenced, and most of the phagotopes thus yielded could be aligned with the Vif sequence. They distributed into four discrete regions in Vif, two of them situated in the central portion of the molecule, between residues T68-L81 and W89-P100, two others at its C-terminus, between residues P162-R167 and P177-M189. They all coincided with regions of the Vif sequence which have a high probability of hydrophilicity and accessibility [177, 178]. One-third of all the Vif-phagotopes isolated were characterized by a high frequency in proline, serine, threonine and basic residues, all features shared by the Vif C-terminus.

Vif Mutants

A natural mutant of Vif, F12-Vif was isolated, where a 45 amino acid region carried six unique amino acids substitutions at positions 127, 128, 130, 131, 132 and 142. This mutant was found to prevent replication and spreading of both CXCR4 and CCR5 strains of HIV-1 in human primary T lymphocyte and T cell lines [179, 180]. T cells transduced with F12-Vif release few HIV-1 virions and with reduced infectivity. Several lines of evidence indicated that HIV-1 interference required the presence of both wild-type and F12-Vif proteins, suggesting a dominant-negative feature of the F12-Vif mutant. The mechanism of F12-Vif mutants has not been reported, however it was found not to depend on the reestablishment of the APOBEC3G function.

Other positions along the Vif sequence (22, 29, 41, 48, 66, 80, 109, 185 and 186) were also found to be important in Vif activity and corresponding Vif mutated sequences were anticipated as possible Vif inhibitors [180].

APOBEC3G Derived Mutants

A gene therapy approach has been proposed based on the finding that a human APOBEC3G containing a single mutation (D128K) could still interact with Vif, but it was not depleted from cells, hence was resistant to Vif activity and inhibited HIV-1 replication in a Vif-resistant manner [152, 181, 182].

Antibody

Engineered Vif-specific single chain intrabodies for gene therapy were also assayed. One of these intrabodies was found to efficiently bind the Vif protein and neutralize its infectivity-enhancing function [183]. Subsequently, this antibody was engineered to mimic camelid antibody domains to improve protein solubility [184]. Indeed, an excellent correlation between improvements in protein solubility with gradually increasing camelization was reported. Camelized single-domains efficiently bound Vif protein and neutralized its infectivity enhancing function, by reducing late reverse transcripts and proviral integration. The activity of the anti-Vif single-domains was shown to be cell-specific, with inhibitory effects only in cells non-permissive that require Vif for HIV-1 replication. Moreover, cell specificity of anti-Vif intrabodies was correlated with an increase of APOBEC3G, which potentiates viral inhibition.

Ribonucleotides (RNAs)

RNA has been successfully used to accomplish Vif inhibition through different strategies.

Initially, a ribozyme specifically targeted against Vif showed efficient cleavage of *vif* RNA; the ribozyme decreased its activity if the hybridization region was either in part deleted or mutated. In the same study, no comparable activity was obtained with a Vif antisense RNA [185].

However, more recently, it was reported that a HIV-1 Vif antisense RNA fragments constructed within the region spanning nucleic acid positions 5561-5705, corresponding to amino acid residues 96-144, significantly inhibited HIV-1 replication in infected cells and reduced the HIV-1 Vif mRNA transcripts [186]. The study demonstrated that virions generated in transfected HeLa-CD4+ cells, especially from HIV-1 Vif frame-shift mutant (3' delta vif; 5561-5849), were affected in splicing and had low infectivity in MT-4 cells [187]. In addition, HIV-1 vif antisense RNA fragments significantly inhibited HIV-1 replication in MT-4 and reduced the HIV-1 *vif* mRNA transcripts and reporter gene (EGFP) expression. The generated virions showed low secondary infection in H9 cells. These data indicate that the middle to the 3' end of Vif is important for its biological activity in the target cells.

Another antisense strategy has been recently applied to inhibit HIV-1 viral replication through *vif* targeting [188]. The antiviral compounds, called peptide-conjugated polymorpholino oligomers (PMO), were composed of the antisense moiety and an arginine rich peptide that increased host cell uptake. The antisense function consisted in a an oligomer or oligonucleotide having a sequence of 12 to 40 morpholino subunits with a *vif* targeting base sequence, linked by partially charged phosphorodiamidate linkages to the peptide fraction.

The preferred target sequence was the region adjacent or including the AUG start site of the Vif gene. The antisense PMO were shown to reduce Vif protein levels and allow

incorporation of APOBEC3G into nascent virions. This substantially reduced the replicative potential of these virions and cause infected cells to produce a virus population with an increased defective to non-defective virion ratio. The overall effect on an *in vivo* infection should be to block the productive infection of lymphoid and myeloid cells and reduce the viral load in the individual.

siRNAs have also been tried against HIV infection. The idea at the base of this strategy is that siRNA, 21-23 mer double-stranded RNAs complementary to a selected RNA sequence, would induce the cleavage of the target mRNA, with consequent protein synthesis inhibition.

A Vif directed siRNA coupled with a HIV-1 decoy TAR RNA [189] was reported. TAR RNA competitively interacts with the HIV-1 Tat protein, to downregulate the enhanced gene expression from the LTR promoter. HIV-1 decoy TAR and Vif siRNA were encoded and expressed in the same construct, and later the TAR and siRNA were cleaved into their respective separate RNA by the endogenous Rnase III-like enzyme. Each of the cleaved HIV-1 anti-genes then synergistically contributed toward enhancing the inhibition efficacy (>80%) of HIV-1 replication in transduced Jurkat cells. These results suggest that targeting HIV-1 mRNA with simultaneously expressed intracellular decoy TAR and Vif-siRNA could lead to an effective gene therapy strategy for the control and management of HIV-AIDS.

However, so far no anti-HIV-1 siRNA-based therapy has reached the clinical trial stage, probably because exact complementarity is a necessity for siRNA activity. Infact, HIV is observed to constantly mutate its genome and this would result in rendering the siRNA-mediated inhibition a failure. An effective alternative strategy maybe the use of miRNAs, which require incomplete complementarity.

In reference [82], human miRNAs targeting HIV-1 genes are presented. In particular, the invention provides targets of six human miRNAs in HIV-1 genome comprising the *nef*, *vpr*, *vif* and *env* genes. Even though the best results were achieved with miRNAs against *nef*, the inventors demonstrated that miRNAs have highly conserved targets in HIV-1 and related late sequences. They are expressed in T-cells, the natural site of infection by HIV-1 infection and their expression level may vary between individuals. Hence, it was shown that human miRNAs have the potential to affect expression of HIV-1 genes and may in future be used to develop therapeutic approaches to inhibit HIV-1.

All these data clearly suggest that Vif could be an effective novel therapeutic target site for gene therapy applications, for the control and management of HIV-1 infection, due to its strong inhibition of HIV-1 replication in cells.

Small Molecules

As seen in the introduction, Vif targets APOBEC3 proteins, cellular antiviral proteins, for proteasomal degradation, in a ubiquitin-dependent process. The Cullin E3 ubiquitin ligases consist of three main components: a Cullin (Cul1,2,3,4a,4b,5, or 7), an adaptor protein, and a substrate receptor [190]. The E3 ligase is the third enzyme in the ubiquitination sequence and is responsible for substrate

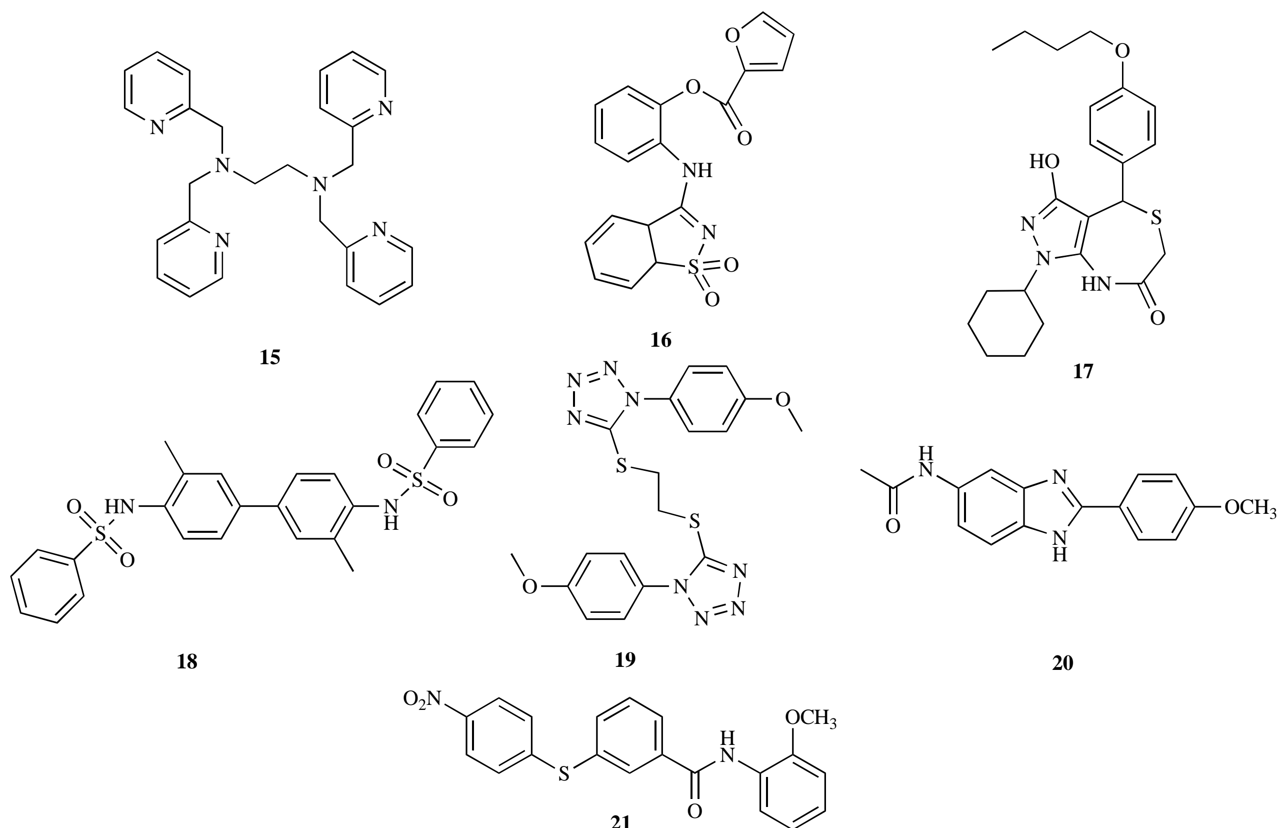

Fig. (9). Chemical structures of small molecules inhibitor of HIV-1 Vif function.

specificity. It has been shown that Vif co-opts the Cullin5 E3 ubiquitin ligase, acting as a substrate receptor, targeting APOBEC3G for proteasomal degradation, and that a highly conserved HCCH zinc binding motif is required for Cul5 selection [191, 192]. Vif specifically interacts with Cul5 through this novel zinc binding motif. It is hence rational to test zinc chelators as inhibitors of HIV-1 Vif-mediated APOBEC3G degradation. Indeed, the membrane-permeable zinc chelator N,N,N',N'-tetrakis-(2-pyridylmethyl) ethylenediamine (TPEN) (compound (**15**), Fig. (**9**)) was reported to increase APOBEC3G stability, allowing it to be packaged within the virion, where it drastically inhibited virus infectivity [193]. Importantly, the TPEN concentrations used (IC_{50} of 1.79 μM) did not affect the function of a number of cellular zinc binding proteins.

A variety of Vif-directed small molecules inhibitors have been recently patented by Rana [194]. These compounds were selected from a small molecule library using a fluorescence based screening method. This method permitted to detect the inhibition of Vif and APOBEC3G and/or APOBEC3F which were covalently linked to fluorescent proteins (i.e. YFP or CFP). The compounds that exhibited the highest inhibitory properties are depicted in Fig. (**9**), compounds (**16**), (**17**), (**18**), (**19**), (**20**). The most promising compounds were also tested for HIV-1 infectivity inhibition and some of them showed encouraging reduction of virus replication.

Among the many tested compounds, some shared a 3-(4-nitrophenylthio)-N-phenylbenzamide template. Of these, compound RN-18 (**21**) (Fig. (**9**)) was shown to antagonize Vif function and inhibit HIV-1 replication only in the presence of APOBEC3G [195]. RN-18 increased cellular APOBEC3G levels in a Vif-dependent manner and increases APOBEC3G incorporation into virions without inhibiting general proteasome-mediated protein degradation. RN-18 enhanced Vif degradation only in the presence of APOBEC3G, reduced viral infectivity by increasing APOBEC3G incorporation into virions and enhances cytidine deamination of the viral genome.

Altogether, these studies provide biochemical evidence for a role of Vif in the HIV-1 lifecycle and validate Vif as an important promising target for the control of HIV-1 infection.

DISCUSSION

Accessory HIV-1 proteins were initially reported to be unnecessary for viral growth in most tissue culture systems; as so they were termed non-essentials and ignored until their importance as virulence factors, governing many interactions between the virus and its host, was understood [196]. As a matter of fact, mutations in all four known HIV-1 accessory proteins, Nef, Vpu, Vpr and Vif, have now been associated with long-term non-progression [87, 106, 197-200].

Three out of four accessory proteins have been involved in CD4 degradation: Nef and Vpr down-regulate CD4 on the cell surface, thereby permitting efficient virus release from the cell membrane; Vpu principal function is the degradation of the CD4/gp160 complex in the ER, thus allowing gp160 recycling and transport to the cell surface for assembly into viral particles. In addition Nef has been implicated in MHC-I down-regulation, and down-modulation of viral infectivity [201, 202]; Vpr is involved in the nuclear transport of the pre-initiation complex (PIC) at the very early steps of viral infection [203, 204], in the induction of G2 cell-cycle arrest in proliferating T limphocytes [205], in the modulation of HIV-1 induced apoptosis [206] and in the expression of Nef [119]. Vif acts during viral assembly in producer cells to ensure infectivity by blocking the potent antiviral function of APOBEC3G [207]. Obviously all these functions play an important role in promoting the virus life cycle, hence the AIDS disease progression. Therefore, being able to suppress accessory protein functions may prove a new potent strategy to contrast HIV-derived disease. However, research against accessory proteins has been so far rather limited. Most importantly, none of the compounds tested *in vitro*, even those that displayed very promising properties, has reached the clinical trials stage.

One main problem encountered in the design of effective drugs against HIV accessory proteins, is the lack of target comprehensive information. In particular, new functions or new interactions are constantly reported, therefore it may be difficult to recognize, and hence target, the primary key functions of the studied accessory protein, against a number of secondary and dispensable effects. Further, structural data have not yet been fully acquired at the NMR level for all accessory proteins, one problem being the extreme flexibility of part of these proteins, which can vary conformation upon interaction with cellular/viral partners. In the case of Vif, structural information is still mostly speculative. The lack of detailed structural data precludes the development of computationally designed ligands. In silico design may be particularly valuable in the development of small molecules, which may be the most effective tools in the search of new antiviral compounds. In fact, small molecule ligands do not need to be structurally related to substrates, hence they could lead to safer and more efficacious drugs by overcoming selectivity and substrate competition issues.

Other approaches, such as those based on peptides/proteins usually present delivery problems, and those based on gene-therapy have not yet been approved due to the potential of severe and irreversible side effects. Si/miRNA strategies could be more realistically applied, even if so far anticancer siRNA have reached the stage of clinical trials, but no siRNA-based drug is yet used in the clinic.

In conclusion, some efficient inhibitors against HIV-1 accessory proteins, especially Nef and Vif, have been described; however, gaining deeper information on the molecular details of HIV-1 accessory protein key functions would trigger the rational development of more effective inhibitors.

In addition, no information is now available on the efficacy of anti-accessory protein compounds in the treatment of AIDS. Hence, verification of the usefulness of these inhibitors used alone or, more likely, in association with other classic antiretroviral drugs is next challenge.

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ABBREVIATIONS

AIDS = acquired immunodeficiency syndrome
AP = adaptor protein
CFP = cyan fluorescent protein
COP = coat protein
CTL = cytotoxic T lymphocytes
EC = effective concentration
EGFP = enhanced green fluorescent protein
ER = endoplasmic reticulum
GPI = glycosylphosphatidylinositol
HAART = highly active antiretroviral therapy
HIV = human immunodeficiency virus
IFN = interferon
IL = interleukin
IN = integrase
LTR = long terminal repeats
MHC = major histocompatibility complex
miRNA = microRNA
NF-kB = nuclear factor kB
NK = natural killer
NLS = nuclear localisation signal
NMR = nuclear magnetic resonance
PACS = phosphofurin acidic cluster sorting protein
PIC = pre-initiation complex
PMO = polymorpholino oligomers
PO-ODNs = phosphodiesters oligonucleotides)
RNAi = RNA interference
RT = reverse transcriptase
siRNA = short interfering RNA
SIV = simian immunodeficiency virus
TCR = T-cell receptor
TM = trans membrane
TNF = tumor necrosis factor
UDG = uracil-DNA glycosylase
YFP = yellow fluorescent protein

REFERENCES

[1] Fauci, A. S.; Macher, A. M.; Longo, D. L.; Lane, H. C.; Rook, A. H.; Masur, H.; Gelmann, E. P. NIH conference. Acquired immunodeficiency syndrome: epidemiologic, clinical, immunologic, and therapeutic considerations. *Ann. Intern. Med.,* **1984**, *100*(1), 92-106.

[2] FDA approves maraviroc tablets. *AIDS Patient Care STDS,* **2007**, *21*(9), 702.

[3] FDA notifications. Accelerated approval for raltegravir tablets. *AIDS Alert,* **2007**, *22*(12), 143-4.

[4] Berrey, M. M.; Schacker, T.; Collier, A. C.; Shea, T.; Brodie, S. J.; Mayers, D.; Coombs, R.; Krieger, J.; Chun, T. W.; Fauci, A.; Self, S. G.; Corey, L. Treatment of primary human immunodeficiency virus type 1 infection with potent antiretroviral therapy reduces frequency of rapid progression to AIDS. *J. Infect. Dis.,* **2001**, *183*(10), 1466-75.

[5] Zhu, T.; Muthui, D.; Holte, S.; Nickle, D.; Feng, F.; Brodie, S.; Hwangbo, Y.; Mullins, J. I.; Corey, L. Evidence for human immunodeficiency virus type 1 replication *in vivo* in CD14(+) monocytes and its potential role as a source of virus in patients on highly active antiretroviral therapy. *J. Virol.,* **2002**, *76*(2), 707-16.

[6] Kinter, A. L.; Umscheid, C. A.; Arthos, J.; Cicala, C.; Lin, Y.; Jackson, R.; Donoghue, E.; Ehler, L.; Adelsberger, J.; Rabin, R. L.; Fauci, A. S. HIV envelope induces virus expression from resting CD4+ T cells isolated from HIV-infected individuals in the absence of markers of cellular activation or apoptosis. *J. Immunol.,* **2003**, *170*(5), 2449-55.

[7] Kumarasamy, N.; Venkatesh, K. K.; Cecelia, A. J.; Devaleenal, B.; Lai, A. R.; Saghayam, S.; Balakrishnan, P.; Yepthomi, T.; Poongulali, S.; Flanigan, T. P.; Solomon, S.; Mayer, K. H. Spectrum of adverse events after generic HAART in southern Indian HIV-infected patients. *AIDS Patient Care STDS,* **2008**, *22*(4), 337-44.

[8] Stevenson, M. HIV-1 pathogenesis. *Nat. Med.,* **2003**, *9*(7), 853-60.

[9] Richter, S. N.; Palu, G. Inhibitors of HIV-1 Tat-mediated transactivation. *Curr. Med. Chem.,* **2006**, *13*(11), 1305-15.

[10] Baba, M. Inhibitors of HIV-1 gene expression and transcription. *Curr. Top. Med. Chem.,* **2004**, *4*(9), 871-82.

[11] Cheng-Mayer, C.; Iannello, P.; Shaw, K.; Luciw, P. A.; Levy, J. A. Differential effects of nef on HIV replication: implications for viral pathogenesis in the host. *Science,* **1989**, *246*(4937), 1629-32.

[12] Miller, M. D.; Feinberg, M. B.; Greene, W. C. The HIV-1 nef gene acts as a positive viral infectivity factor. *Trends Microbiol.,* **1994**, *2*(8), 294-8.

[13] Miller, M. D.; Warmerdam, M. T.; Gaston, I.; Greene, W. C.; Feinberg, M. B. The human immunodeficiency virus-1 nef gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. *J. Exp. Med.,* **1994**, *179*(1), 101-13.

[14] Learmont, J. C.; Geczy, A. F.; Mills, J.; Ashton, L. J.; Raynes-Greenow, C. H.; Garsia, R. J.; Dyer, W. B.; McIntyre, L.; Oelrichs, R. B.; Rhodes, D. I.; Deacon, N. J.; Sullivan, J. S. Immunologic and virologic status after 14 to 18 years of infection with an attenuated strain of HIV-1. A report from the Sydney Blood Bank Cohort. *N. Engl. J. Med.,* **1999**, *340*(22), 1715-22.

[15] Salvi, R.; Garbuglia, A. R.; Di Caro, A.; Pulciani, S.; Montella, F.; Benedetto, A. Grossly defective nef gene sequences in a human immunodeficiency virus type 1-seropositive long-term nonprogressor. *J. Virol.,* **1998**, *72*(5), 3646-57.

[16] Kestler, H. W., 3rd; Ringler, D. J.; Mori, K.; Panicali, D. L.; Sehgal, P. K.; Daniel, M. D.; Desrosiers, R. C. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. *Cell,* **1991**, *65*(4), 651-62.

[17] Geyer, M.; Fackler, O. T.; Peterlin, B. M. Structure--function relationships in HIV-1 Nef. *EMBO Rep.,* **2001**, *2*(7), 580-5.

[18] Das, S. R.; Jameel, S. Biology of the HIV Nef protein. *Indian J. Med. Res.,* **2005**, *121*(4), 315-32.

[19] Geyer, M.; Munte, C. E.; Schorr, J.; Kellner, R.; Kalbitzer, H. R. Structure of the anchor-domain of myristoylated and non-myristoylated HIV-1 Nef protein. *J. Mol. Biol.,* **1999**, *289*(1), 123-38.

[20] Arold, S. T.; Baur, A. S. Dynamic Nef and Nef dynamics: how structure could explain the complex activities of this small HIV protein. *Trends Biochem. Sci.,* **2001**, *26*(6), 356-63.

[21] Arien, K. K.; Verhasselt, B. HIV Nef: role in pathogenesis and viral fitness. *Curr. HIV Res.,* **2008**, *6*(3), 200-8.

[22] Joseph, A. M.; Kumar, M.; Mitra, D. Nef: "necessary and enforcing factor" in HIV infection. *Curr. HIV Res.,* **2005**, *3*(1), 87-94.

[23] Kirchhoff, F.; Schindler, M.; Specht, A.; Arhel, N.; Munch, J. Role of Nef in primate lentiviral immunopathogenesis. *Cell Mol. Life Sci.,* **2008**, *65*(17), 2621-36.

[24] Munch, J.; Rajan, D.; Schindler, M.; Specht, A.; Rucker, E.; Novembre, F. J.; Nerrienet, E.; Muller-Trutwin, M. C.; Peeters, M.; Hahn, B. H.; Kirchhoff, F. Nef-mediated enhancement of virion in-

fectivity and stimulation of viral replication are fundamental properties of primate lentiviruses. *J. Virol.,* **2007**, *81*(24), 13852-64.

[25] Rauch, S.; Pulkkinen, K.; Saksela, K.; Fackler, O. T. Human immunodeficiency virus type 1 Nef recruits the guanine exchange factor Vav1 *via* an unexpected interface into plasma membrane microdomains for association with p21-activated kinase 2 activity. *J. Virol.,* **2008**, *82*(6), 2918-29.

[26] Agopian, K.; Wei, B. L.; Garcia, J. V.; Gabuzda, D. A hydrophobic binding surface on the human immunodeficiency virus type 1 Nef core is critical for association with p21-activated kinase 2. *J. Virol.,* **2006**, *80*(6), 3050-61.

[27] Lu, X.; Wu, X.; Plemenitas, A.; Yu, H.; Sawai, E. T.; Abo, A.; Peterlin, B. M. CDC42 and Rac1 are implicated in the activation of the Nef-associated kinase and replication of HIV-1. *Curr. Biol.,* **1996**, *6*(12), 1677-84.

[28] Raney, A.; Shaw, A. Y.; Foster, J. L.; Garcia, J. V. Structural constraints on human immunodeficiency virus type 1 Nef function. *Virology,* **2007**, *368*(1), 7-16.

[29] Trible, R. P.; Emert-Sedlak, L.; Smithgall, T. E. HIV-1 Nef selectively activates Src family kinases Hck, Lyn, and c-Src through direct SH3 domain interaction. *J. Biol. Chem.,* **2006**, *281*(37), 27029-38.

[30] Fackler, O. T.; Baur, A. S. Live and let die: Nef functions beyond HIV replication. *Immunity,* **2002**, *16*(4), 493-7.

[31] Roeth, J. F.; Williams, M.; Kasper, M. R.; Filzen, T. M.; Collins, K. L. HIV-1 Nef disrupts MHC-I trafficking by recruiting AP-1 to the MHC-I cytoplasmic tail. *J. Cell. Biol.,* **2004**, *167*(5), 903-13.

[32] Williams, M.; Roeth, J. F.; Kasper, M. R.; Fleis, R. I.; Przybycin, C. G.; Collins, K. L. Direct binding of human immunodeficiency virus type 1 Nef to the major histocompatibility complex class I (MHC-I) cytoplasmic tail disrupts MHC-I trafficking. *J. Virol.,* **2002**, *76*(23), 12173-84.

[33] Bentham, M.; Mazaleyrat, S.; Harris, M. The di-leucine motif in the cytoplasmic tail of CD4 is not required for binding to human immunodeficiency virus type 1 Nef, but is critical for CD4 down-modulation. *J. Gen. Virol.,* **2003**, *84*(Pt 10), 2705-13.

[34] Giolo, G.; Neri, F.; Casartelli, N.; Potesta, M.; Belleudi, F.; Torrisi, M. R.; Doria, M. Internalization and intracellular retention of CD4 are two separate functions of the human immunodeficiency virus type 1 Nef protein. *J. Gen. Virol.,* **2007**, *88*(Pt 11), 3133-8.

[35] Pizzato, M.; Helander, A.; Popova, E.; Calistri, A.; Zamborlini, A.; Palu, G.; Gottlinger, H. G. Dynamin 2 is required for the enhancement of HIV-1 infectivity by Nef. *Proc. Natl. Acad. Sci. USA,* **2007**, *104*(16), 6812-7.

[36] Schindler, M.; Munch, J.; Kutsch, O.; Li, H.; Santiago, M. L.; Bibollet-Ruche, F.; Muller-Trutwin, M. C.; Novembre, F. J.; Peeters, M.; Courgnaud, V.; Bailes, E.; Roques, P.; Sodora, D. L.; Silvestri, G.; Sharp, P. M.; Hahn, B. H.; Kirchhoff, F. Nef-mediated suppression of T cell activation was lost in a lentiviral lineage that gave rise to HIV-1. *Cell,* **2006**, *125*(6), 1055-67.

[37] O'Neill, E.; Kuo, L. S.; Krisko, J. F.; Tomchick, D. R.; Garcia, J. V.; Foster, J. L. Dynamic evolution of the human immunodeficiency virus type 1 pathogenic factor, Nef. *J. Virol.,* **2006**, *80*(3), 1311-20.

[38] Roeth, J. F.; Collins, K. L. Human immunodeficiency virus type 1 Nef: adapting to intracellular trafficking pathways. *Microbiol. Mol. Biol. Rev.,* **2006**, *70*(2), 548-63.

[39] Le Gall, S.; Erdtmann, L.; Benichou, S.; Berlioz-Torrent, C.; Liu, L.; Benarous, R.; Heard, J. M.; Schwartz, O. Nef interacts with the mu subunit of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC I molecules. *Immunity,* **1998**, *8*(4), 483-95.

[40] Piguet, V.; Wan, L.; Borel, C.; Mangasarian, A.; Demaurex, N.; Thomas, G.; Trono, D. HIV-1 Nef protein binds to the cellular protein PACS-1 to downregulate class I major histocompatibility complexes. *Nat. Cell. Biol.,* **2000**, *2*(3), 163-7.

[41] Schwartz, O.; Marechal, V.; Le Gall, S.; Lemonnier, F.; Heard, J. M. Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. *Nat. Med.,* **1996**, *2*(3), 338-42.

[42] Blagoveshchenskaya, A. D.; Thomas, L.; Feliciangeli, S. F.; Hung, C. H.; Thomas, G. HIV-1 Nef downregulates MHC-I by a PACS-1- and PI3K-regulated ARF6 endocytic pathway. *Cell,* **2002**, *111*(6), 853-66.

[43] Faure, J.; Stalder, R.; Borel, C.; Sobo, K.; Piguet, V.; Demaurex, N.; Gruenberg, J.; Trono, D. ARF1 regulates Nef-induced CD4 degradation. *Curr. Biol.,* **2004**, *14*(12), 1056-64.

[44] Craig, H. M.; Pandori, M. W.; Guatelli, J. C. Interaction of HIV-1 Nef with the cellular dileucine-based sorting pathway is required for CD4 down-regulation and optimal viral infectivity. *Proc. Natl. Acad. Sci. USA,* **1998**, *95*(19), 11229-34.

[45] Geyer, M.; Yu, H.; Mandic, R.; Linnemann, T.; Zheng, Y. H.; Fackler, O. T.; Peterlin, B. M. Subunit H of the V-ATPase binds to the medium chain of adaptor protein complex 2 and connects Nef to the endocytic machinery. *J. Biol. Chem.,* **2002**, *277*(32), 28521-9.

[46] Baur, A. S.; Sawai, E. T.; Dazin, P.; Fantl, W. J.; Cheng-Mayer, C.; Peterlin, B. M. HIV-1 Nef leads to inhibition or activation of T cells depending on its intracellular localization. *Immunity,* **1994**, *1*(5), 373-84.

[47] Fackler, O. T.; Luo, W.; Geyer, M.; Alberts, A. S.; Peterlin, B. M. Activation of Vav by Nef induces cytoskeletal rearrangements and downstream effector functions. *Mol. Cell.,* **1999**, *3*(6), 729-39.

[48] Skowronski, J.; Parks, D.; Mariani, R. Altered T cell activation and development in transgenic mice expressing the HIV-1 nef gene. *EMBO J.,* **1993**, *12*(2), 703-13.

[49] Fackler, O. T.; Lu, X.; Frost, J. A.; Geyer, M.; Jiang, B.; Luo, W.; Abo, A.; Alberts, A. S.; Peterlin, B. M. p21-activated kinase 1 plays a critical role in cellular activation by Nef. *Mol. Cell. Biol.,* **2000**, *20*(7), 2619-27.

[50] Nunn, M. F.; Marsh, J. W. Human immunodeficiency virus type 1 Nef associates with a member of the p21-activated kinase family. *J. Virol.,* **1996**, *70*(9), 6157-61.

[51] Vilhardt, F.; Plastre, O.; Sawada, M.; Suzuki, K.; Wiznerowicz, M.; Kiyokawa, E.; Trono, D.; Krause, K. H. The HIV-1 Nef protein and phagocyte NADPH oxidase activation. *J. Biol. Chem.,* **2002**, *277*(44), 42136-43.

[52] Janardhan, A.; Swigut, T.; Hill, B.; Myers, M. P.; Skowronski, J. HIV-1 Nef binds the DOCK2-ELMO1 complex to activate rac and inhibit lymphocyte chemotaxis. *PLoS Biol.,* **2004**, *2*(1), E6.

[53] Xu, X. N.; Laffert, B.; Screaton, G. R.; Kraft, M.; Wolf, D.; Kolanus, W.; Mongkolsapay, J.; McMichael, A. J.; Baur, A. S. Induction of Fas ligand expression by HIV involves the interaction of Nef with the T cell receptor zeta chain. *J. Exp. Med.,* **1999**, *189*(9), 1489-96.

[54] Geleziunas, R.; Xu, W.; Takeda, K.; Ichijo, H.; Greene, W. C. HIV-1 Nef inhibits ASK1-dependent death signalling providing a potential mechanism for protecting the infected host cell. *Nature,* **2001**, *410*(6830), 834-8.

[55] Greenway, A. L.; McPhee, D. A.; Allen, K.; Johnstone, R.; Holloway, G.; Mills, J.; Azad, A.; Sankovich, S.; Lambert, P. Human immunodeficiency virus type 1 Nef binds to tumor suppressor p53 and protects cells against p53-mediated apoptosis. *J. Virol.,* **2002**, *76*(6), 2692-702.

[56] Wolf, D.; Witte, V.; Laffert, B.; Blume, K.; Stromer, E.; Trapp, S.; d'Aloja, P.; Schurmann, A.; Baur, A. S. HIV-1 Nef associated PAK and PI3-kinases stimulate Akt-independent Bad-phosphorylation to induce anti-apoptotic signals. *Nat. Med.,* **2001**, *7*(11), 1217-24.

[57] Benichou, S.; Bomsel, M.; Bodeus, M.; Durand, H.; Doute, M.; Letourneur, F.; Camonis, J.; Benarous, R. Physical interaction of the HIV-1 Nef protein with beta-COP, a component of non-clathrin-coated vesicles essential for membrane traffic. *J. Biol. Chem.,* **1994**, *269*(48), 30073-6.

[58] Janvier, K.; Craig, H.; Hitchin, D.; Madrid, R.; Sol-Foulon, N.; Renault, L.; Cherfils, J.; Cassel, D.; Benichou, S.; Guatelli, J. HIV-1 Nef stabilizes the association of adaptor protein complexes with membranes. *J. Biol. Chem.,* **2003**, *278*(10), 8725-32.

[59] Chaudhry, A.; Das, S. R.; Hussain, A.; Mayor, S.; George, A.; Bal, V.; Jameel, S.; Rath, S. The Nef protein of HIV-1 induces loss of cell surface costimulatory molecules CD80 and CD86 in APCs. *J. Immunol.,* **2005**, *175*(7), 4566-74.

[60] Stove, V.; Van de Walle, I.; Naessens, E.; Coene, E.; Stove, C.; Plum, J.; Verhasselt, B. Human immunodeficiency virus Nef induces rapid internalization of the T-cell coreceptor CD8alphabeta. *J. Virol.,* **2005**, *79*(17), 11422-33.

[61] Vigerust, D. J.; Egan, B. S.; Shepherd, V. L. HIV-1 Nef mediates post-translational down-regulation and redistribution of the mannose receptor. *J. Leukoc. Biol.,* **2005**, *77*(4), 522-34.

[62] Bewley, C. A.; Ray, S.; Cohen, F.; Collins, S. K.; Overman, L. E. Inhibition of HIV-1 envelope-mediated fusion by synthetic batzelladine analogues. *J. Nat. Prod.,* **2004**, *67*(8), 1319-24.
[63] Chang, L.; Whittaker, N. F.; Bewley, C. A. Crambescidin 826 and dehydrocrambine A: new polycyclic guanidine alkaloids from the marine sponge Monanchora sp. that inhibit HIV-1 fusion. *J. Nat. Prod.,* **2003**, *66*(11), 1490-4.
[64] Olszewski, A.; Sato, K.; Aron, Z. D.; Cohen, F.; Harris, A.; McDougall, B. R.; Robinson, W. E., Jr.; Overman, L. E.; Weiss, G. A. Guanidine alkaloid analogs as inhibitors of HIV-1 Nef interactions with p53, actin, and p56lck. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(39), 14079-84.
[65] Olszewski, A.; Weiss, G. A. Library versus library recognition and inhibition of the HIV-1 Nef allelome. *J. Am. Chem. Soc.,* **2005**, *127*(35), 12178-9.
[66] Arold, S.; O'Brien, R.; Franken, P.; Strub, M. P.; Hoh, F.; Dumas, C.; Ladbury, J. E. RT Loop Flexibility Enhances the Specificity of Src Family SH3 Domains for HIV-1 Nef. *Biochemistry,* **1998**, *37*(42), 14683-14691.
[67] Hanna, Z.; Weng, X.; Kay, D. G.; Poudrier, J.; Lowell, C.; Jolicoeur, P. The pathogenicity of human immunodeficiency virus (HIV) type 1 Nef in CD4C/HIV transgenic mice is abolished by mutation of its SH3-binding domain, and disease development is delayed in the absence of Hck. *J. Virol.,* **2001**, *75*(19), 9378-92.
[68] Betzi, S.; Restouin, A.; Opi, S.; Arold, S. T.; Parrot, I.; Guerlesquin, F.; Morelli, X.; Collette, Y. Protein protein interaction inhibition (2P2I) combining high throughput and virtual screening: Application to the HIV-1 Nef protein. *Proc. Natl. Acad. Sci. USA,* **2007**, *104*(49), 19256-61.
[69] Murakami, Y.; Fukazawa, H.; Kobatake, T.; Yamagoe, S.; Takebe, Y.; Tobiume, M.; Matsuda, M.; Uehara, Y. A mammalian two-hybrid screening system for inhibitors of interaction between HIV Nef and the cellular tyrosine kinase Hck. *Antiviral. Res.,* **2002**, *55*(1), 161-8.
[70] Luo, T.; Anderson, S. J.; Garcia, J. V. Inhibition of Nef- and phorbol ester-induced CD4 degradation by macrolide antibiotics. *J. Virol.,* **1996**, *70*(3), 1527-34.
[71] Jacque, J. M.; Triques, K.; Stevenson, M. Modulation of HIV-1 replication by RNA interference. *Nature,* **2002**, *418*(6896), 435-8.
[72] Novina, C. D.; Murray, M. F.; Dykxhoorn, D. M.; Beresford, P. J.; Riess, J.; Lee, S. K.; Collman, R. G.; Lieberman, J.; Shankar, P.; Sharp, P. A. siRNA-directed inhibition of HIV-1 infection. *Nat. Med.,* **2002**, *8*(7), 681-6.
[73] Das, A. T.; Brummelkamp, T. R.; Westerhout, E. M.; Vink, M.; Madiredjo, M.; Bernards, R.; Berkhout, B. Human immunodeficiency virus type 1 escapes from RNA interference-mediated inhibition. *J. Virol.,* **2004**, *78*(5), 2601-5.
[74] Dave, R. S.; Pomerantz, R. J. Antiviral effects of human immunodeficiency virus type 1-specific small interfering RNAs against targets conserved in select neurotropic viral strains. *J. Virol.,* **2004**, *78*(24), 13687-96.
[75] Omoto, S.; Ito, M.; Tsutsumi, Y.; Ichikawa, Y.; Okuyama, H.; Brisibe, E. A.; Saksena, N. K.; Fujii, Y. R. HIV-1 nef suppression by virally encoded microRNA. *Retrovirology,* **2004**, *1*, 44.
[76] Yamamoto, T.; Miyoshi, H.; Yamamoto, N.; Yamamoto, N.; Inoue, J.; Tsunetsugu-Yokota, Y. Lentivirus vectors expressing short hairpin RNAs against the U3-overlapping region of HIV nef inhibit HIV replication and infectivity in primary macrophages. *Blood,* **2006**, *108*(10), 3305-12.
[77] Westerhout, E. M.; Ooms, M.; Vink, M.; Das, A. T.; Berkhout, B. HIV-1 can escape from RNA interference by evolving an alternative structure in its RNA genome. *Nucleic Acids Res.,* **2005**, *33*(2), 796-804.
[78] ter Brake, O.; Konstantinova, P.; Ceylan, M.; Berkhout, B. Silencing of HIV-1 with RNA interference: a multiple shRNA approach. *Mol. Ther.,* **2006**, *14*(6), 883-92.
[79] Konstantinova, P.; ter Brake, O.; Haasnoot, J.; de Haan, P.; Berkhout, B. Trans-inhibition of HIV-1 by a long hairpin RNA expressed within the viral genome. *Retrovirology,* **2007**, *4*, 15.
[80] Yamamoto, T.; Omoto, S.; Mizuguchi, M.; Mizukami, H.; Okuyama, H.; Okada, N.; Saksena, N. K.; Brisibe, E. A.; Otake, K.; Fuji, Y. R. Double-stranded nef RNA interferes with human immunodeficiency virus type 1 replication. *Microbiol. Immunol.,* **2002**, *46*(11), 809-17.
[81] Hariharan, M.; Scaria, V.; Pillai, B.; Brahmachari, S. K. Targets for human encoded microRNAs in HIV genes. *Biochem. Biophys. Res. Commun.,* **2005**, *337*(4), 1214-8.
[82] Brahmachari, S. K.; Hariharan, M. J. Human microRNA targets in HIV genome and a method of identification thereof. US2007087335, 2007-04-19, 2007.
[83] Hiipakka, M.; Huotari, P.; Manninen, A.; Renkema, G. H.; Saksela, K. Inhibition of cellular functions of HIV-1 Nef by artificial SH3 domains. *Virology,* **2001**, *286*(1), 152-9.
[84] Stangler, T.; Tran, T.; Hoffmann, S.; Schmidt, H.; Jonas, E.; Willbold, D. Competitive displacement of full-length HIV-1 Nef from the Hck SH3 domain by a high-affinity artificial peptide. *Biol. Chem.,* **2007**, *388*(6), 611-5.
[85] Hout, D. R.; Mulcahy, E. R.; Pacyniak, E.; Gomez, L. M.; Gomez, M. L.; Stephens, E. B. Vpu: a multifunctional protein that enhances the pathogenesis of human immunodeficiency virus type 1. *Curr. HIV Res.,* **2004**, *2*(3), 255-70.
[86] Fischer, W. B. Vpu from HIV-1 on an atomic scale: experiments and computer simulations. *FEBS Lett.,* **2003**, *552*(1), 39-46.
[87] Stephens, E. B.; McCormick, C.; Pacyniak, E.; Griffin, D.; Pinson, D. M.; Sun, F.; Nothnick, W.; Wong, S. W.; Gunderson, R.; Berman, N. E.; Singh, D. K. Deletion of the vpu sequences prior to the env in a simian-human immunodeficiency virus results in enhanced Env precursor synthesis but is less pathogenic for pig-tailed macaques. *Virology,* **2002**, *293*(2), 252-61.
[88] Bour, S.; Boulerice, F.; Wainberg, M. A. Inhibition of gp160 and CD4 maturation in U937 cells after both defective and productive infections by human immunodeficiency virus type 1. *J. Virol.,* **1991**, *65*(12), 6387-96.
[89] Bour, S.; Schubert, U.; Strebel, K. The human immunodeficiency virus type 1 Vpu protein specifically binds to the cytoplasmic domain of CD4: implications for the mechanism of degradation. *J. Virol.,* **1995**, *69*(3), 1510-20.
[90] Margottin, F.; Benichou, S.; Durand, H.; Richard, V.; Liu, L. X.; Gomas, E.; Benarous, R. Interaction between the cytoplasmic domains of HIV-1 Vpu and CD4: role of Vpu residues involved in CD4 interaction and *in vitro* CD4 degradation. *Virology,* **1996**, *223*(2), 381-6.
[91] Maldarelli, F.; Chen, M. Y.; Willey, R. L.; Strebel, K. Human immunodeficiency virus type 1 Vpu protein is an oligomeric type I integral membrane protein. *J. Virol.,* **1993**, *67*(8), 5056-61.
[92] Bour, S.; Strebel, K. The HIV-1 Vpu protein: a multifunctional enhancer of viral particle release. *Microbes Infect.,* **2003**, *5*(11), 1029-39.
[93] Deora, A.; Spearman, P.; Ratner, L. The N-terminal matrix domain of HIV-1 Gag is sufficient but not necessary for viral protein U-mediated enhancement of particle release through a membrane-targeting mechanism. *Virology,* **2000**, *269*(2), 305-12.
[94] Montal, M. Structure-function correlates of Vpu, a membrane protein of HIV-1. *FEBS Lett.,* **2003**, *552*(1), 47-53.
[95] Nomaguchi, M.; Fujita, M.; Adachi, A. Role of HIV-1 Vpu protein for virus spread and pathogenesis. *Microbes Infect.,* **2008**, *10*(9), 960-7
[96] Ewart, G. D.; Mills, K.; Cox, G. B.; Gage, P. W. Amiloride derivatives block ion channel activity and enhancement of virus-like particle budding caused by HIV-1 protein Vpu. *Eur. Biophys. J.,* **2002**, *31*(1), 26-35.
[97] Premkumar, A.; Wilson, L.; Ewart, G. D.; Gage, P. W. Cation-selective ion channels formed by p7 of hepatitis C virus are blocked by hexamethylene amiloride. *FEBS Lett.,* **2004**, *557*(1-3), 99-103.
[98] Kleyman, T. R.; Cragoe, E. J., Jr. Cation transport probes: the amiloride series. *Methods Enzymol.,* **1990**, *191*, 739-55.
[99] Lemaitre, V.; Ali, R.; Kim, C. G.; Watts, A.; Fischer, W. B. Interaction of amiloride and one of its derivatives with Vpu from HIV-1: a molecular dynamics simulation. *FEBS Lett.,* **2004**, *563*(1-3), 75-81.
[100] Lemaitre, V.; Willbold, D.; Watts, A.; Fischer, W. B. Full length Vpu from HIV-1: combining molecular dynamics simulations with NMR spectroscopy. *J. Biomol. Struct. Dyn.,* **2006**, *23*(5), 485-96.
[101] Waheed, A. A.; Ablan, S. D.; Soheilian, F.; Nagashima, K.; Ono, A.; Schaffner, C. P.; Freed, E. O. Inhibition of human immunodeficiency virus type 1 assembly and release by the cholesterol-binding

compound amphotericin B methyl ester: evidence for Vpu dependence. *J. Virol.,* **2008**, *82*(19), 9776-81.

[102] Neil, S. J.; Zang, T.; Bieniasz, P. D. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature,* **2008**, *451*(7177), 425-30.

[103] Neil, S. J.; Sandrin, V.; Sundquist, W. I.; Bieniasz, P. D. An interferon-alpha-induced tethering mechanism inhibits HIV-1 and Ebola virus particle release but is counteracted by the HIV-1 Vpu protein. *Cell Host Microbe,* **2007**, *2*(3), 193-203.

[104] Rollason, R.; Korolchuk, V.; Hamilton, C.; Schu, P.; Banting, G. Clathrin-mediated endocytosis of a lipid-raft-associated protein is mediated through a dual tyrosine motif. *J. Cell. Sci.,* **2007**, *120*(Pt 21), 3850-8.

[105] Khamsri, B.; Murao, F.; Yoshida, A.; Sakurai, A.; Uchiyama, T.; Shirai, H.; Matsuo, Y.; Fujita, M.; Adachi, A. Comparative study on the structure and cytopathogenic activity of HIV Vpr/Vpx proteins. *Microbes Infect.,* **2006**, *8*(1), 10-5.

[106] Lum, J. J.; Cohen, O. J.; Nie, Z.; Weaver, J. G.; Gomez, T. S.; Yao, X. J.; Lynch, D.; Pilon, A. A.; Hawley, N.; Kim, J. E.; Chen, Z.; Montpetit, M.; Sanchez-Dardon, J.; Cohen, E. A.; Badley, A. D. Vpr R77Q is associated with long-term nonprogressive HIV infection and impaired induction of apoptosis. *J. Clin. Invest.,* **2003**, *111*(10), 1547-54.

[107] Gibbs, J. S.; Lackner, A. A.; Lang, S. M.; Simon, M. A.; Sehgal, P. K.; Daniel, M. D.; Desrosiers, R. C. Progression to AIDS in the absence of a gene for vpr or vpx. *J. Virol.,* **1995**, *69*(4), 2378-83.

[108] Morellet, N.; Bouaziz, S.; Petitjean, P.; Roques, B. P. NMR structure of the HIV-1 regulatory protein VPR. *J. Mol. Biol.,* **2003**, *327*(1), 215-27.

[109] Nermut, M. V.; Fassati, A. Structural analyses of purified human immunodeficiency virus type 1 intracellular reverse transcription complexes. *J. Virol.,* **2003**, *77*(15), 8196-206.

[110] Stark, L. A.; Hay, R. T. Human immunodeficiency virus type 1 (HIV-1) viral protein R (Vpr) interacts with Lys-tRNA synthetase: implications for priming of HIV-1 reverse transcription. *J. Virol.,* **1998**, *72*(4), 3037-44.

[111] Mansky, L. M.; Preveral, S.; Selig, L.; Benarous, R.; Benichou, S. The interaction of vpr with uracil DNA glycosylase modulates the human immunodeficiency virus type 1 *in vivo* mutation rate. *J. Virol.,* **2000**, *74*(15), 7039-47.

[112] Le Rouzic, E.; Mousnier, A.; Rustum, C.; Stutz, F.; Hallberg, E.; Dargemont, C.; Benichou, S. Docking of HIV-1 Vpr to the nuclear envelope is mediated by the interaction with the nucleoporin hCG1. *J. Biol. Chem.,* **2002**, *277*(47), 45091-8.

[113] Jacotot, E.; Ravagnan, L.; Loeffler, M.; Ferri, K. F.; Vieira, H. L.; Zamzami, N.; Costantini, P.; Druillennec, S.; Hoebeke, J.; Briand, J. P.; Irinopoulou, T.; Daugas, E.; Susin, S. A.; Cointe, D.; Xie, Z. H.; Reed, J. C.; Roques, B. P.; Kroemer, G. The HIV-1 viral protein R induces apoptosis *via* a direct effect on the mitochondrial permeability transition pore. *J. Exp. Med.,* **2000**, *191*(1), 33-46.

[114] Roumier, T.; Vieira, H. L.; Castedo, M.; Ferri, K. F.; Boya, P.; Andreau, K.; Druillennec, S.; Joza, N.; Penninger, J. M.; Roques, B.; Kroemer, G. The C-terminal moiety of HIV-1 Vpr induces cell death *via* a caspase-independent mitochondrial pathway. *Cell Death Differ.,* **2002**, *9*(11), 1212-9.

[115] Sabbah, E. N.; Druillennec, S.; Morellet, N.; Bouaziz, S.; Kroemer, G.; Roques, B. P. Interaction between the HIV-1 protein Vpr and the adenine nucleotide translocator. *Chem. Biol. Drug Des.,* **2006**, *67*(2), 145-54.

[116] Jacotot, E.; Ferri, K. F.; El Hamel, C.; Brenner, C.; Druillennec, S.; Hoebeke, J.; Rustin, P.; Metivier, D.; Lenoir, C.; Geuskens, M.; Vieira, H. L.; Loeffler, M.; Belzacq, A. S.; Briand, J. P.; Zamzami, N.; Edelman, L.; Xie, Z. H.; Reed, J. C.; Roques, B. P.; Kroemer, G. Control of mitochondrial membrane permeabilization by adenine nucleotide translocator interacting with HIV-1 viral protein rR and Bcl-2. *J. Exp. Med.,* **2001**, *193*(4), 509-19.

[117] Levy, D. N.; Refaeli, Y.; Weiner, D. B. Extracellular Vpr protein increases cellular permissiveness to human immunodeficiency virus replication and reactivates virus from latency. *J. Virol.,* **1995**, *69*(2), 1243-52.

[118] Sawaya, B. E.; Khalili, K.; Mercer, W. E.; Denisova, L.; Amini, S. Cooperative actions of HIV-1 Vpr and p53 modulate viral gene transcription. *J. Biol. Chem.,* **1998**, *273*(32), 20052-7.

[119] Poon, B.; Chang, M. A.; Chen, I. S. Vpr is required for efficient Nef expression from unintegrated human immunodeficiency virus type 1 DNA. *J. Virol.,* **2007**, *81*(19), 10515-23.

[120] Le Rouzic, E.; Benichou, S. The Vpr protein from HIV-1: distinct roles along the viral life cycle. *Retrovirology,* **2005**, *2*, 11.

[121] Watanabe, N.; Nishihara, Y.; Yamaguchi, T.; Koito, A.; Miyoshi, H.; Kakeya, H.; Osada, H. Fumagillin suppresses HIV-1 infection of macrophages through the inhibition of Vpr activity. *FEBS Lett.,* **2006**, *580*(11), 2598-602.

[122] Kamata, M.; Wu, R. P.; An, D. S.; Saxe, J. P.; Damoiseaux, R.; Phelps, M. E.; Huang, J.; Chen, I. S. Cell-based chemical genetic screen identifies damnacanthal as an inhibitor of HIV-1 Vpr induced cell death. *Biochem. Biophys. Res. Commun.,* **2006**, *348*(3), 1101-6.

[123] Nishizawa, M.; Kamata, M.; Mojin, T.; Nakai, Y.; Aida, Y. Induction of apoptosis by the Vpr protein of human immunodeficiency virus type 1 occurs independently of G(2) arrest of the cell cycle. *Virology,* **2000**, *276*(1), 16-26.

[124] Poon, B.; Jowett, J. B.; Stewart, S. A.; Armstrong, R. W.; Rishton, G. M.; Chen, I. S. Human immunodeficiency virus type 1 vpr gene induces phenotypic effects similar to those of the DNA alkylating agent, nitrogen mustard. *J. Virol.,* **1997**, *71*(5), 3961-71.

[125] Sukhija, R.; Yalamanchili, K.; Aronow, W. S.; Kakar, P.; Babu, S. Clinical characteristics, risk factors, and medical treatment of 561 patients with peripheral arterial disease followed in an academic vascular surgery clinic. *Cardiol. Rev.,* **2005**, *13*(2), 108-10.

[126] Rodriguez-Moran, M.; Guerrero-Romero, F. Efficacy of pentoxifylline in the management of microalbuminuria in patients with diabetes. *Curr. Diabetes Rev.,* **2008**, *4*(1), 55-62.

[127] Smith, J. A.; Nunnari, G.; Preuss, M.; Pomerantz, R. J.; Daniel, R. Pentoxifylline suppresses transduction by HIV-1-based vectors. *Intervirology,* **2007**, *50*(5), 377-86.

[128] Tondelli, L.; Colonna, F. P.; Garbesi, A.; Zanella, S.; Marongiu, M. E.; Corrias, S.; Loi, A. G.; La Colla, P. Native oligodeoxynucleotides specifically active against human immunodeficiency virus type 1 *in vitro*: a G-quartet-driven effect? *Antimicrob. Agents Chemother.,* **1996**, *40*(9), 2034-8.

[129] Yao, X. J.; Lemay, J.; Rougeau, N.; Clement, M.; Kurtz, S.; Belhumeur, P.; Cohen, E. A. Genetic selection of peptide inhibitors of human immunodeficiency virus type 1 Vpr. *J. Biol. Chem.,* **2002**, *277*(50), 48816-26.

[130] BouHamdan, M.; Xue, Y.; Baudat, Y.; Hu, B.; Sire, J.; Pomerantz, R. J.; Duan, L. X. Diversity of HIV-1 Vpr interactions involves usage of the WXXF motif of host cell proteins. *J. Biol. Chem.,* **1998**, *273*(14), 8009-16.

[131] Gleenberg, I. O.; Herschhorn, A.; Hizi, A. Inhibition of the activities of reverse transcriptase and integrase of human immunodeficiency virus type-1 by peptides derived from the homologous viral protein R (Vpr). *J. Mol. Biol.,* **2007**, *369*(5), 1230-43.

[132] Sawaya, B. E.; Khalili, K.; Rappaport, J.; Serio, D.; Chen, W.; Srinivasan, A.; Amini, S. Suppression of HIV-1 transcription and replication by a Vpr mutant. *Gene Ther.,* **1999**, *6*(5), 947-50.

[133] Kino, T.; Gragerov, A.; Kopp, J. B.; Stauber, R. H.; Pavlakis, G. N.; Chrousos, G. P. The HIV-1 virion-associated protein vpr is a coactivator of the human glucocorticoid receptor. *J. Exp. Med.,* **1999**, *189*(1), 51-62.

[134] BouHamdan, M.; Kulkosky, J.; Duan, L. X.; Pomerantz, R. J. Inhibition of HIV-1 replication and infectivity by expression of a fusion protein, VPR-anti-integrase single-chain variable fragment (SFv): intravirion molecular therapies. *J. Hum. Virol.,* **2000**, *3*(1), 6-15.

[135] Serio, D.; Rizvi, T. A.; Cartas, M.; Kalyanaraman, V. S.; Weber, I. T.; Koprowski, H.; Srinivasan, A. Development of a novel anti-HIV-1 agent from within: effect of chimeric Vpr-containing protease cleavage site residues on virus replication. *Proc. Natl. Acad. Sci. USA,* **1997**, *94*(7), 3346-51.

[136] Wu, X.; Liu, H.; Xiao, H.; Kappes, J. C. Proteolytic activity of human immunodeficiency virus Vpr- and Vpx-protease fusion proteins. *Virology,* **1996**, *219*(1), 307-13.

[137] Wu, X.; Liu, H.; Xiao, H.; Conway, J. A.; Kappes, J. C. Inhibition of human and simian immunodeficiency virus protease function by targeting Vpx-protease-mutant fusion protein into viral particles. *J. Virol.,* **1996**, *70*(6), 3378-84.

[138] Sova, P.; van Ranst, M.; Gupta, P.; Balachandran, R.; Chao, W.; Itescu, S.; McKinley, G.; Volsky, D. J. Conservation of an intact human immunodeficiency virus type 1 vif gene *in vitro* and *in vivo*. *J. Virol.,* **1995**, *69*(4), 2557-64.

[139] Paul, I.; Cui, J.; Maynard, E. L. Zinc binding to the HCCH motif of HIV-1 virion infectivity factor induces a conformational change that mediates protein-protein interactions. *Proc. Natl. Acad. Sci. USA,* **2006**, *103*(49), 18475-80.

[140] Khan, M. A.; Aberham, C.; Kao, S.; Akari, H.; Gorelick, R.; Bour, S.; Strebel, K. Human immunodeficiency virus type 1 Vif protein is packaged into the nucleoprotein complex through an interaction with viral genomic RNA. *J. Virol,* **2001**, *75*(16), 7252-65.

[141] Goncalves, J.; Shi, B.; Yang, X.; Gabuzda, D. Biological activity of human immunodeficiency virus type 1 Vif requires membrane targeting by C-terminal basic domains. *J. Virol.,* **1995**, *69*(11), 7196-204.

[142] Bouyac, M.; Courcoul, M.; Bertoia, G.; Baudat, Y.; Gabuzda, D.; Blanc, D.; Chazal, N.; Boulanger, P.; Sire, J.; Vigne, R.; Spire, B. Human immunodeficiency virus type 1 Vif protein binds to the Pr55Gag precursor. *J. Virol.,* **1997**, *71*(12), 9358-65.

[143] Donahue, J. P.; Vetter, M. L.; Mukhtar, N. A.; D'Aquila, R. T. The HIV-1 Vif PPLP motif is necessary for human APOBEC3G binding and degradation. *Virology,* **2008**, *377*(1), 49-53.

[144] Yang, S.; Sun, Y.; Zhang, H. The multimerization of human immunodeficiency virus type I Vif protein: a requirement for Vif function in the viral life cycle. *J. Biol. Chem.,* **2001**, *276*(7), 4889-93.

[145] Sheehy, A. M.; Gaddis, N. C.; Choi, J. D.; Malim, M. H. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature,* **2002**, *418*(6898), 646-50.

[146] Cullen, B. R. Role and mechanism of action of the APOBEC3 family of antiretroviral resistance factors. *J. Virol.,* **2006**, *80*(3), 1067-76.

[147] Turelli, P.; Mangeat, B.; Jost, S.; Vianin, S.; Trono, D. Inhibition of hepatitis B virus replication by APOBEC3G. *Science,* **2004**, *303*(5665), 1829.

[148] Mangeat, B.; Turelli, P.; Caron, G.; Friedli, M.; Perrin, L.; Trono, D. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature,* **2003**, *424*(6944), 99-103.

[149] Harris, R. S.; Bishop, K. N.; Sheehy, A. M.; Craig, H. M.; Petersen-Mahrt, S. K.; Watt, I. N.; Neuberger, M. S.; Malim, M. H. DNA deamination mediates innate immunity to retroviral infection. *Cell,* **2003**, *113*(6), 803-9.

[150] Bogerd, H. P.; Doehle, B. P.; Wiegand, H. L.; Cullen, B. R. A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(11), 3770-4.

[151] Schrofelbauer, B.; Chen, D.; Landau, N. R. A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(11), 3927-32.

[152] Xu, H.; Svarovskaia, E. S.; Barr, R.; Zhang, Y.; Khan, M. A.; Strebel, K.; Pathak, V. K. A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(15), 5652-7.

[153] Mangeat, B.; Turelli, P.; Liao, S.; Trono, D. A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. *J. Biol. Chem.,* **2004**, *279*(15), 14481-3.

[154] Strebel, K. HIV accessory genes Vif and Vpu. *Adv. Pharmacol.,* **2007**, *55*, 199-232.

[155] Mariani, R.; Chen, D.; Schrofelbauer, B.; Navarro, F.; Konig, R.; Bollman, B.; Munk, C.; Nymark-McMahon, H.; Landau, N. R. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell,* **2003**, *114*(1), 21-31.

[156] Sheehy, A. M.; Gaddis, N. C.; Malim, M. H. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat. Med.,* **2003**, *9*(11), 1404-7.

[157] Mehle, A.; Strack, B.; Ancuta, P.; Zhang, C.; McPike, M.; Gabuzda, D. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. *J. Biol. Chem.,* **2004**, *279*(9), 7792-8.

[158] Yu, X.; Yu, Y.; Liu, B.; Luo, K.; Kong, W.; Mao, P.; Yu, X. F. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science,* **2003**, *302*(5647), 1056-60.

[159] Yu, Y.; Xiao, Z.; Ehrlich, E. S.; Yu, X.; Yu, X. F. Selective assembly of HIV-1 Vif-Cul5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cysteines. *Genes Dev.,* **2004**, *18*(23), 2867-72.

[160] Camaur, D.; Trono, D. Characterization of human immunodeficiency virus type 1 Vif particle incorporation. *J. Virol.,* **1996**, *70*(9), 6106-11.

[161] Karczewski, M. K.; Strebel, K. Cytoskeleton association and virion incorporation of the human immunodeficiency virus type 1 Vif protein. *J. Virol.,* **1996**, *70*(1), 494-507.

[162] Hassaine, G.; Courcoul, M.; Bessou, G.; Barthalay, Y.; Picard, C.; Olive, D.; Collette, Y.; Vigne, R.; Decroly, E. The tyrosine kinase Hck is an inhibitor of HIV-1 replication counteracted by the viral vif protein. *J. Biol. Chem.,* **2001**, *276*(20), 16885-93.

[163] Lake, J. A.; Carr, J.; Feng, F.; Mundy, L.; Burrell, C.; Li, P. The role of Vif during HIV-1 infection: interaction with novel host cellular factors. *J. Clin. Virol.,* **2003**, *26*(2), 143-52.

[164] Feng, F.; Davis, A.; Lake, J. A.; Carr, J.; Xia, W.; Burrell, C.; Li, P. Ring finger protein ZIN interacts with human immunodeficiency virus type 1 Vif. *J. Virol.,* **2004**, *78*(19), 10574-81.

[165] Chen, D.; Li, X.; Zhai, Z.; Shu, H. B. A novel zinc finger protein interacts with receptor-interacting protein (RIP) and inhibits tumor necrosis factor (TNF)- and IL1-induced NF-kappa B activation. *J. Biol. Chem.,* **2002**, *277*(18), 15985-91.

[166] Casero, R. A., Jr.; Celano, P.; Ervin, S. J.; Applegren, N. B.; Wiest, L.; Pegg, A. E. Isolation and characterization of a cDNA clone that codes for human spermidine/spermine N1-acetyltransferase. *J. Biol. Chem.,* **1991**, *266*(2), 810-4.

[167] Casero, R.A. Jr.; Pegg, A.E. Spermidine/spermine N1-acetyl-transferase--the turning point in polyamine metabolism. *FASEB J.,* **1993**, *7*(8), 653-61.

[168] Madani, N.; Millette, R.; Platt, E. J.; Marin, M.; Kozak, S. L.; Bloch, D. B.; Kabat, D. Implication of the lymphocyte-specific nuclear body protein Sp140 in an innate response to human immunodeficiency virus type 1. *J. Virol.,* **2002**, *76*(21), 11133-8.

[169] Bloch, D. B.; Chiche, J. D.; Orth, D.; de la Monte, S. M.; Rosenzweig, A.; Bloch, K. D. Structural and functional heterogeneity of nuclear bodies. *Mol. Cell. Biol.,* **1999**, *19*(6), 4423-30.

[170] Mezei, M.; Minarovits, J. Reversal of HIV drug resistance and novel strategies to curb HIV infection: the viral infectivity factor Vif as a target and tool of therapy. *Curr. Drug. Targets,* **2006**, *7*(7), 881-5.

[171] Reeves, J. D.; Piefer, A. J. Emerging drug targets for antiretroviral therapy. *Drugs,* **2005**, *65*(13), 1747-66.

[172] Bovolenta, C. Blocking HIV-1 Vif restores a natural mechanism of intracellular antiviral defense. *Curr. Drug Targets Immune Endocr. Metabol. Disord.,* **2004**, *4*(4), 257-63.

[173] Carr, J. M.; Davis, A. J.; Feng, F.; Burrell, C. J.; Li, P. Cellular interactions of virion infectivity factor (Vif) as potential therapeutic targets: APOBEC3G and more? *Curr. Drug Targets,* **2006**, *7*(12), 1583-93.

[174] Yang, B.; Gao, L.; Li, L.; Lu, Z.; Fan, X.; Patel, C. A.; Pomerantz, R. J.; DuBois, G. C.; Zhang, H. Potent suppression of viral infectivity by the peptides that inhibit multimerization of human immunodeficiency virus type 1 (HIV-1) Vif proteins. *J. Biol. Chem.,* **2003**, *278*(8), 6596-602.

[175] Zhang, H.; Pomerantz, R. J.; Yang, B. Multimerization of HIV-1 Vif protein as a therapeutic target. AU2002307229B, 2007-05-24, 2007.

[176] Zhang, H.; Pomerantz, R. J.; Yang, B. Multimerization of HIV-1 VIF protein as a therapeutic target. US2004086512, 2004-05-06, 2004.

[177] Wieland, U.; Kratschmann, H.; Kehm, R.; Kuhn, J. E.; Naher, H.; Kramer, M. D.; Braun, R. W. Antigenic domains of the HIV-1 vif protein as recognized by human sera and murine monoclonal antibodies. *AIDS Res. Hum. Retroviruses,* **1991**, *7*(11), 861-7.

[178] Wieland, U.; Hartmann, J.; Suhr, H.; Salzberger, B.; Eggers, H. J.; Kuhn, J. E. *In vivo* genetic variability of the HIV-1 vif gene. *Virology,* **1994**, *203*(1), 43-51.

[179] Vallanti, G.; Lupo, R.; Federico, M.; Mavilio, F.; Bovolenta, C. T Lymphocytes transduced with a lentiviral vector expressing F12-

Vif are protected from HIV-1 infection in an APOBEC3G-independent manner. *Mol. Ther.,* **2005**, *12*(4), 697-706.
[180] Bovolenta, C. HIV Vif mutants. CA2599525, 2006-10-26 2006.
[181] Santa-Marta, M.; da Silva, F. A.; Fonseca, A. M.; Goncalves, J. HIV-1 Vif can directly inhibit apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G-mediated cytidine deamination by using a single amino acid interaction and without protein degradation. *J. Biol. Chem.,* **2005**, *280*(10), 8765-75.
[182] Pathak, V. K.; Xu, H.; Svarovskaia, E. S.; Barr, R. Reduction of HIV-1 replication by a mutant apolipoprotein B mRNA editing enzyme-catalytic polypeptide-like (APOBEC3G). WO2005077099 2005-08-25, 2005.
[183] Goncalves, J.; Silva, F.; Freitas-Vieira, A.; Santa-Marta, M.; Malho, R.; Yang, X.; Gabuzda, D.; Barbas, C. 3rd. Functional neutralization of HIV-1 Vif protein by intracellular immunization inhibits reverse transcription and viral replication. *J. Biol. Chem.,* **2002**, *277*(35), 32036-45.
[184] Aires da Silva, F.; Santa-Marta, M.; Freitas-Vieira, A.; Mascarenhas, P.; Barahona, I.; Moniz-Pereira, J.; Gabuzda, D.; Goncalves, J. Camelized rabbit-derived VH single-domain intrabodies against Vif strongly neutralize HIV-1 infectivity. *J. Mol. Biol.,* **2004**, *340*(3), 525-42.
[185] Lorentzen, E. U.; Wieland, U.; Kuhn, J. E.; Braun, R. W. *In vitro* cleavage of HIV-1 vif RNA by a synthetic ribozyme. *Virus Genes,* **1991**, *5*(1), 17-23.
[186] Barnor, J. S.; Miyano-Kurosaki, N.; Yamaguchi, K.; Sakamoto, A.; Ishikawa, K.; Inagaki, Y.; Yamamoto, N.; Osei-Kwasi, M.; Ofori-Adjei, D.; Takaku, H. Intracellular expression of antisense RNA transcripts complementary to the human immunodeficiency virus type-1 vif gene inhibits viral replication in infected T-lymphoblastoid cells. *Biochem. Biophys. Res. Commun.,* **2004**, *320*(2), 544-50.
[187] Barnor, J. S.; Miyano-Kurosaki, N.; Takaku, H.; Yamaguchi, K.; Sakamoto, A.; Ishikawa, K.; Yamamoto, N.; Osei-Kwasi, M.; Ofori-Adjei, D. The middle to 3' end of the HIV-1 vif gene sequence is important for vif biological activity and could be used for antisense oligonucleotide targets. *Nucleosides Nucleotides Nucleic Acids,* **2005**, *24*(10-12), 1745-61.
[188] Mourich, D. V.; Iversen, P. L.; Bestwick, R. K.; Weller, D. D. Method and antisense composition for selective inhibition of HIV infection in hematopoietic cells. US2007037764, 2007-02-15, 2007.
[189] Barnor, J. S.; Miyano-Kurosaki, N.; Yamaguchi, K.; Abumi, Y.; Ishikawa, K.; Yamamoto, N. Lentiviral-mediated delivery of combined HIV-1 decoy TAR and Vif siRNA as a single RNA molecule that cleaves to inhibit HIV-1 in transduced cells. *Nucleosides Nucleotides Nucleic Acids,* **2005**, *24*(5-7), 431-4.
[190] Skowyra, D.; Craig, K. L.; Tyers, M.; Elledge, S. J.; Harper, J. W. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell,* **1997**, *91*(2), 209-19.
[191] Luo, K.; Xiao, Z.; Ehrlich, E.; Yu, Y.; Liu, B.; Zheng, S.; Yu, X. F. Primate lentiviral virion infectivity factors are substrate receptors that assemble with cullin 5-E3 ligase through a HCCH motif to suppress APOBEC3G. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*(32), 11444-9.
[192] Xiao, Z.; Ehrlich, E.; Yu, Y.; Luo, K.; Wang, T.; Tian, C.; Yu, X. F. Assembly of HIV-1 Vif-Cul5 E3 ubiquitin ligase through a novel zinc-binding domain-stabilized hydrophobic interface in Vif. *Virology,* **2006**, *349*(2), 290-9.
[193] Xiao, Z.; Ehrlich, E.; Luo, K.; Xiong, Y.; Yu, X. F. Zinc chelation inhibits HIV Vif activity and liberates antiviral function of the cytidine deaminase APOBEC3G. *FASEB J.,* **2007**, *21*(1), 217-22.
[194] Rana, T. M. Composition ond synthesis of new reagents for inhibition of HIV replication. WO2007044565 2007-04-19, 2007.
[195] Nathans, R.; Cao, H.; Sharova, N.; Ali, A.; Sharkey, M.; Stranska, R.; Stevenson, M.; Rana, T. M. Small-molecule inhibition of HIV-1 Vif. *Nat. Biotechnol.,* **2008**, *26*(10), 1187-92.
[196] Trono, D. When accessories turn out to be essential. *Nat. Med.,* **1998**, *4*(12), 1368-9.
[197] Kirchhoff, F.; Greenough, T. C.; Brettler, D. B.; Sullivan, J. L.; Desrosiers, R. C. Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. *N. Engl. J. Med.,* **1995**, *332*(4), 228-32.
[198] Deacon, N. J.; Tsykin, A.; Solomon, A.; Smith, K.; Ludford-Menting, M.; Hooker, D. J.; McPhee, D. A.; Greenway, A. L.; Ellett, A.; Chatfield, C.; Lawson, V. A.; Crowe, S.; Maerz, A.; Sonza, S.; Learmont, J.; Sullivan, J. S.; Cunningham, A.; Dwyer, D.; Dowton, D.; Mills, J. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. *Science,* **1995**, *270*(5238), 988-91.
[199] Mariani, R.; Kirchhoff, F.; Greenough, T. C.; Sullivan, J. L.; Desrosiers, R. C.; Skowronski, J. High frequency of defective nef alleles in a long-term survivor with nonprogressive human immunodeficiency virus type 1 infection. *J. Virol.,* **1996**, *70*(11), 7752-64.
[200] Alexander, L.; Aquino-DeJesus, M. J.; Chan, M.; Andiman, W. A. Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by a two-amino-acid insertion in HIV-1 Vif from a nonprogressing mother and child. *J. Virol.,* **2002**, *76*(20), 10533-9.
[201] Collins, K. L.; Chen, B. K.; Kalams, S. A.; Walker, B. D.; Baltimore, D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature,* **1998**, *391*(6665), 397-401.
[202] Swann, S. A.; Williams, M.; Story, C. M.; Bobbitt, K. R.; Fleis, R.; Collins, K. L. HIV-1 Nef blocks transport of MHC class I molecules to the cell surface *via* a PI 3-kinase-dependent pathway. *Virology,* **2001**, *282*(2), 267-77.
[203] Fouchier, R. A.; Meyer, B. E.; Simon, J. H.; Fischer, U.; Albright, A. V.; Gonzalez-Scarano, F.; Malim, M. H. Interaction of the human immunodeficiency virus type 1 Vpr protein with the nuclear pore complex. *J. Virol.,* **1998**, *72*(7), 6004-13.
[204] Hrimech, M.; Yao, X. J.; Bachand, F.; Rougeau, N.; Cohen, E. A. Human immunodeficiency virus type 1 (HIV-1) Vpr functions as an immediate-early protein during HIV-1 infection. *J. Virol.,* **1999**, *73*(5), 4101-9.
[205] Poon, B.; Grovit-Ferbas, K.; Stewart, S. A.; Chen, I. S. Cell cycle arrest by Vpr in HIV-1 virions and insensitivity to antiretroviral agents. *Science,* **1998**, *281*(5374), 266-9.
[206] Muthumani, K.; Hwang, D. S.; Desai, B. M.; Zhang, D.; Dayes, N.; Green, D. R.; Weiner, D. B. HIV-1 Vpr induces apoptosis through caspase 9 in T cells and peripheral blood mononuclear cells. *J. Biol. Chem.,* **2002**, *277*(40), 37820-31.
[207] Simon, J. H.; Carpenter, E. A.; Fouchier, R. A.; Malim, M. H. Vif and the p55(Gag) polyprotein of human immunodeficiency virus type 1 are present in colocalizing membrane-free cytoplasmic complexes. *J. Virol.,* **1999**, *73*(4), 2667-74.
[208] Strebel, K. HIV-1 Vpu: putting a channel to the TASK. *Mol. Cell.,* **2004**, *14*(2), 150-2.
[209] Lv, W.; Liu, Z.; Jin, H.; Yu, X.; Zhang, L.; Zhang, L. Three-dimensional structure of HIV-1 VIF constructed by comparative modeling and the function characterization analyzed by molecular dynamics simulation. *Org. Biomol. Chem.,* **2007**, *5*(4), 617-26.

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