HIV Structural Biology, Current Treatment, Application of Gene Editing Technology, Stem Cells Based Therapies and Broadly Neutralizing Antibodies For HIV Eradication

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Abstract- *The HIV-1 pandemic is a complex mix of diverse epidemics within and between countries and regions of the world, and is undoubtedly the defining public-health crisis of our time. Research has deepened our understanding of how the virus replicates, manipulates, and hides in an infected person. Although our understanding of pathogenesis and transmission dynamics has become more nuanced and prevention options have expanded, a cure or protective vaccine remains elusive. Antiretroviral treatment has transformed AIDS from an inevitably fatal condition to a chronic, manageable disease in some settings. The field of HIV cure research has seen major breakthroughs with the success of the first phase I clinical trial involving gene editing of CCR5 in patient-derived CD4+ T cells. This first human use of gene editing technology was accomplished using zinc-finger nucleases (ZFNs). ZFNs and the advent of additional tools for genome engineering, including TALENS and the CRISPR/Cas9 system, have made gene editing remarkably simple and affordable. Hematopoietic stem cells can self renew, proliferate and differentiate into mature immune cells. In theory, anti-HIV gene modified hematopoietic stem cells can continuously provide HIV resistant immune cells throughout the life of a patient. Therefore, hematopoietic stem cell based anti-HIV gene therapy has a great potential to provide a life-long remission of HIV/AIDS by a single treatment. Recent advances have led to the discovery of a large portfolio of human monoclonal antibodies that are broadly neutralizing across many HIV-1 subtypes and are also substantially more potent. These antibodies target multiple different epitopes on the HIV envelope, thus allowing for the development of antibody combinations.*

Keywords- CCR5, cell antiviral factor (Caf), CRISPR/Cas9, TALENs, Zinc Finger Nucleases (ZFN),Broadly neutralizing antibodies (bNAbs)

I. INTRODUCTION

An estimated 38·6 million people live with HIV-1 worldwide, while about 25 million have died already. These estimates mask the dynamic nature of this evolving epidemic in relation to temporal changes, geographic distribution, magnitude, viral diversity, and mode of transmission. Today, there is no region of the world untouched by this pandemic.Based on their genetic make-up, HIV-1 viruses are divided into three groups (eg, M [main], N, and O group). These HIV-1 groups and HIV-2 probably result from distinct cross-species transmission events. Pandemic HIV-1 has diversified into at least nine subtypes and many circulating recombinant forms, which encode genetic structures from two or more subtypes (eg, A/E=CRF01; A/G=CRF02). Current enzyme immunoassays are sensitive enough to detect antibody as early as one to two weeks after infection. A variety of other assays are essential to confirm positive antibody screens (Western blot, polymerase chain reaction [PCR]), provide an adjunct to antibody testing (p24 antigen, PCR), or provide additional information for the clinician treating HIV-positive patients (qualitative and quantitative PCR, and genotyping).As highly active antiretroviral therapy (HAART) transforms human immune deficiency virus (HIV) into a manageable chronic disease, new challenges are emerging in treating children born with HIV, including a number of risks to their physical and psychological health due to HIV infection and its lifelong treatment.The story of Timothy Brown, better known as the Berlin Patient, is an optimistic one for the community. Brown, suffering from acute myeloid leukemia and HIV-1 infection, required a bone marrow transplant and was given donor cells from an individual homozygous for a naturally occurring 32bp deletion in CCR5, an HIV-1 co-receptor required for entry. Following the success of the transplant, Brown discontinued HIV-1 therapy and has no detectable viremia for over six years. From this observation, CCR5 modification has become a gene therapy target for curative HIV research and the logic of stem cells based therapies for

HIV treatment came up.The principal idea of anti-HIV hematopoietic stem/progenitor cell (HSPC) based gene therapy is to genetically engineer patient-derived HSPC and progenies to resist HIV infection because HIV resistant genetically engineered HSPC, in theory, can provide HIV resistant progenies continuously through an entire life of a patient with a single treatment.The recent discovery of highly potent, broadly neutralizing, HIV-specific monoclonal antibodies (bNAbs) provides a novel class of potential therapeutic agents. It has long been known that neutralizing antibodies can target the HIV envelope (Env) and effectively suppress viral replication in vitro. New developments in high throughput single-cell BCR amplification and novel soluble Env selection tools have led to the isolation of new monoclonal antibodies with substantially increased potency and breadth. Moreover, preclinical data in the non-human primate model using the V3 glycan-dependent bNAb PGT121 demonstrated reduction of proviral DNA in both blood and tissues.

II. STRUCTURE AND LIFE CYCLE OF HIV

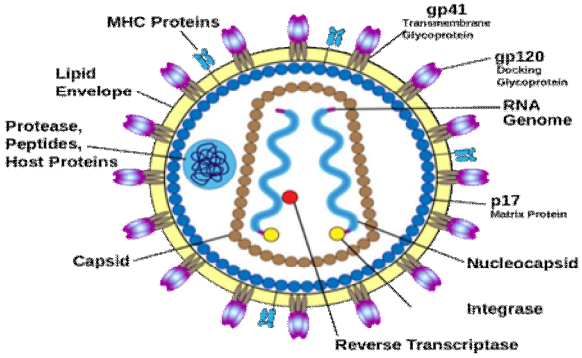

Figure 1 Structure of HIV

HIV is an enveloped RNA virus. As HIV buds out of the host cell during replication, it acquires a phospholipid envelope. Protruding from the envelope are peg-like structures that the viral RNA encodes. Each peg consists of three or four gp41 glycoproteins (the stem), capped with three or four gp120 glycoproteins. Inside the envelope the bullet shaped nucleocapsid of the virus is composed of protein and surrounds two single strands of RNA. Three enzymes important to the virus's life cycle — reverse transcriptase, integrase, and protease — are also within the nucleocapsid. Reverse transcriptase allows the single-stranded RNA of the virus to be copied and double-stranded DNA (dsDNA) to be generated. The enzyme integrase then facilitates the integration of this viral DNA into the cellular chromosome. Although helper T cells seem to be the main target for HIV, other cells can become infected as well. These include monocytes and macrophages, which can hold large numbers of viruses within themselves without being killed. Some T cells harbour similar reservoirs of the virus. Entry of HIV into the host cell requires the binding of one or more gp120 molecules on the virus to CD4 molecules on the host cell's surface. Binding to a second receptor is also required. One, CCR5, a chemokine receptor, serves as a co receptor early in an infection. Another chemokine receptor (CXCR4) later serves as a co receptor. Viruses isolated from individuals early in an infection, during the asymptomatic phase, will typically infect macrophages in the laboratory, but not T cells (the viruses are Mtropic). Virus isolated from patients later in the infection, in the symptomatic phase, will infect T cells (the viruses are T-tropic). It seems that a shift takes place in the viral population during the progression of the infection so that new cellular receptors are used and different cells become infected. Provirus (HIV DNA) is replicated along with the chromosome when the cell divides. The integration of provirus into the host DNA provides the latency that enables the virus to evade host responses so effectively. Production of viral proteins and RNA takes place when the provirus is transcribed. Viral proteins are then assembled using the host cell's protein-making machinery. The virus's protease enzyme allows for the processing of newly translated polypeptides into the proteins, which are then ultimately assembled into viral particles. The virus eventually buds out of the cell. A cell infected with a retrovirus does not necessarily lyse the cell when viral replication takes place; rather, many viral particles can bud out of a cell over the course of time.

III. EFFECT OF HIV ON BODY

A. BODY'S RESPONSE

A.1 Humoral Response

Antibodies to HIV can be detected soon after acute infection, often as early a few days after exposure to the virus, but generally within 1 to 3 months. These antibodies can be found in the blood, on mucosal surfaces, and in various body fluids. IgG1 is a key player in host defence at all stages of infection, and helps to combat the virus with antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and neutralizing and blocking responses. Antibody-dependent cellular cytotoxicity is directed against sites on the viral envelope, especially against sites on the V3 loop and the extracellular domain of gp41. All the other antibody isotypes (IgM, IgA, IgG2, IGG4 and IGD) vary in their levels throughout the course of infection, and there is no known correlation between isotype appearance and stage of disease. Neutralizing antibodies offer some protection by limiting viral replication during the early asymptomatic stages, but overall their titers tend to remain too low to clear the HIV

infection. The low titer is probably a result of the fact that the virus's envelope epitopes have a highly dynamic configuration, which changes often, depending on the state of activation and binding to cellular receptors. The low titer also favours the emergence of resistant mutants during active replication.

A.2 Cell-Mediated Immune Responses

Antigen-specific cellular immune responses include the work of cytotoxic T cells (CTLs), and Helper-T cell activities that stimulate B cells and cytotoxic lymphocytes. Most cytotoxic activity is carried out by CD8+ cells, which produce a cell antiviral factor (Caf), which can suppress viral gene transcription. CTL activity is directed against antigens on a variety of HIV proteins, including envelope glycoproteins, and internal structural proteins (p17, p24, p15), as well as against the products of regulatory genes such as nef and vif and pol. CTLs are capable of lysing cells infected with HIV and have the advantage of a broad-based activity against isolates from different clades. CTL activity induced by HIV-2 can also lyse cells infected with HIV-1.

A.3Deleterious Immune Responses

Some antibodies called "interfering antibodies" can bind to virions or infected cells and block interaction with neutralizing antibodies. Others called "enhancing antibodies" actually facilitate infection by allowing virions coated with them to enter cells. These immune responses are of concern in efforts to develop HIV-1 vaccines, but so far autoimmunity, immune suppression, enhancement of infection and other deleterious effects have not been encountered in vaccines trials to date.

B. STAGES OF INFECTION (TIME LINE)-

B.1 Acute Primary Infection

During the acute primary infection phase, some HIV-infected cells have begun to circulate through the blood system. However, the immune system has not been damaged badly yet, so it responds by producing HIV antibodies and with the help of all the uninfected T-cells, it mounts an attack two to four weeks after infection. At this time, HIV levels in the blood will be greatly reduced.

B.2 Clinical Latent Infection

HIV is infiltrating the body and attacking its defence system. While levels of the virus in the blood may be low, nearly undetectable in some cases, HIV has become extremely active in the body's lymph system. Untreated, this second stage of an HIV infection has an average duration of 10 years.

B.3 Symptomatic HIV Infection

Over time, the infection advances. When the body's viral load — a measurement of how many cells in the blood are infected with HIV — begins rising to higher and higher levels, this indicates the immune system is deteriorating. In this phase, opportunistic infections, which would not harm a healthy person, become likely and make an HIV patient sick.

B.4 Progression from HIV to AIDS

In the final stage of HIV, a patient's T-cell count falls as viral load increases — the immune system becomes severely compromised. When the patient is diagnosed with a stage-4 HIV-related condition such as tuberculosis or cancer or pneumonia, the virus has made its progression to AIDS, Acquired Immunodeficiency Syndrome.

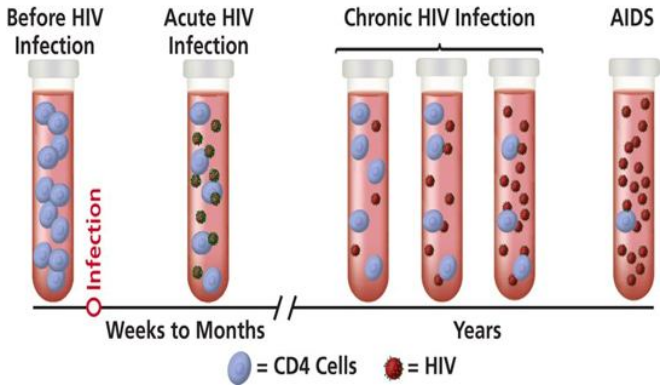

Figure 2 HIV progression

IV. DRUG CATEGORIES, SIDE EFFECTS AND LIFE EXPECTANCY

Anti-HIV drugs are also called antiretroviral drugs or antiretroviral (ARVs). They work because they attack the HIV virus directly. The drugs cripple the ability of the virus to make copies of itself.

There are 6 main classes of anti-HIV drugs:

A. Nucleoside Reverse Transcriptase Inhibitors (NRTIs or nukes)

When the HIV virus enters a healthy cell, it attempts to make copies of itself. It does this by using an enzyme called reverse transcriptase. The NRTIs work because they block that enzyme. Without reverse transcriptase, HIV can't make new virus copies of itself. Eg. Zaiden, Combivir, Videx

B.Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs or non-nukes)

NNRTIs, also known as "non-nucleosides" or "non-nukes" for short, attach themselves to reverse transcriptase and prevent the enzyme from converting RNA to DNA. In turn, HIV's genetic material cannot be incorporated into the healthy genetic material of the cell, and prevents the cell from producing new virus. Eg. Edurant, Sustiva

C. Protease Inhibitors (PIs)

Once HIV has infected a cell and made copies of itself, it uses an enzyme called protease to process itself correctly so it can be released from the cell to infect other cells. These medicines work by blocking protease. Eg. Agenerase, Reyaztaz

D. Integrase Inhibitors

This class of anti-HIV drugs works by blocking an enzyme (HIV integrase) that the virus needs in order to splice copies of itself into human DNA. Eg. Isentress, Tivicay

E. Fusion or Entry Inhibitors

The fusion or entry inhibitors work by stopping the HIV virus from getting into your body's healthy cells in the first place. Eg. Fuzeon,Selzentry

F. Chemokine Co-Receptor Antagonists (CCR5)

To infect a cell, HIV must bind to two types of molecules on the cell's surface. One of these is called a chemokine co-receptor. Drugs known as chemokine co-receptor antagonists block the virus from binding to the co-receptor.Ex-Tybost

V. GENOME EDITING TECHNOLOGY

Current therapy using anti-HIV drugs inhibits reverse transcription, integration, maturation, and entry steps of HIV replication described as a circle (○) (Left, the target of HIV drugs is indicated as a blue character). However, those drugs are ineffective for latently HIV-infected cells described as a cross mark (x). Genome-editing technology is able to distinguish the latently-infected and uninfected cells by the genome sequence. In uninfected cells, CCR5 or other host factors can be targeted by the genome-editing technology (Right).

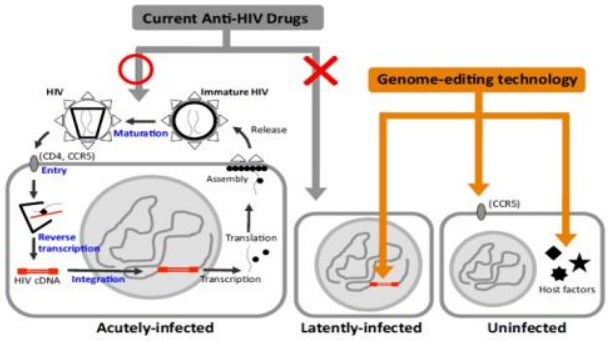

Figure 3 Current Anti HIV drugs and Genome editing Technology

A. Strategies of nuclease-based HIV therapy targeting HIV provirus

TAR region in LTRs are designed as target of CRISPR/Cas9 and TALENs. Designable nucleases targeted TAR is able to inactivate the LTR promoter and excise the internal sequence of HIV provirus.

If nuclease-based HIV therapy is utilized for clinical applications, the goal will be to reduce off-target mutations and obtain higher nuclease activity. TALEN technology may be a better choice for the application because they exhibit significantly reduced off-target effects and cytotoxicity.

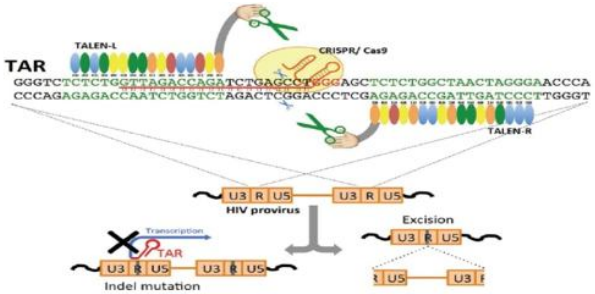

Figure 4 Nuclease-based HIV therapy

B. Inhibition of HIV replication with anti-HIV nuclease

HIV cDNA is proposed to be integrated at the host genome interacting with nuclear pore complex immediately after nuclear import. If HIV cDNA is cleaved before integration, HIV replication can be inhibited.

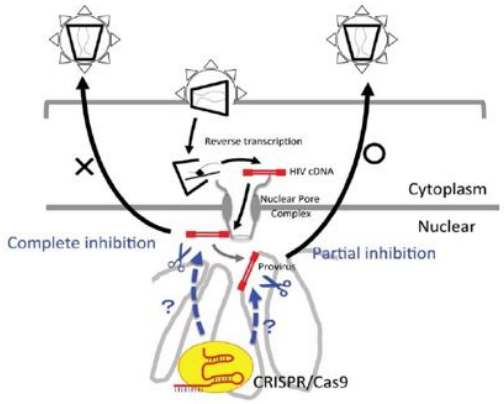

Figure 5 Anti HIV Nuclease

C. Shock and Kill strategy using genome-editing technology.

The CRISPR/dCas9-VP64 system can be used as an HIV promoter-specific activator.

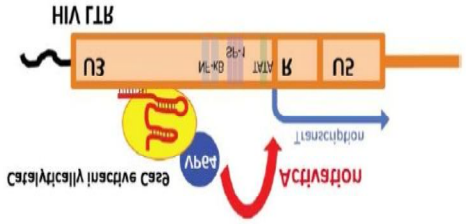

Figure 6 Shock and Kill strategy

VI. STEM CELLS BASED THERAPIES FOR HIV/AIDS

Hematopoietic stem cell based anti-HIV gene therapy aims to reconstitute patient immune system by transplantation of genetically engineered hematopoietic stem cells with anti-HIV genes. Hematopoietic stem cells can self renew, proliferate and differentiate into mature immune cells. In theory, anti-HIV gene modified hematopoietic stem cells can continuously provide HIV resistant immune cells throughout the life of a patient. Therefore, hematopoietic stem cell based anti-HIV gene therapy has a great potential to provide a life-long remission of HIV/AIDS by a single treatment.

The principal idea of anti-HIV hematopoietic stem/progenitor cell (HSPC) based gene therapy is to genetically engineer patient-derived HSPC and progenies to resist HIV infection. HIV resistant genetically engineered HSPC, in theory, can provide HIV resistant progenies continuously through an entire life of a patient with a single treatment.

Anti HIV genes provide resistance.

- *A. Targeting the viral entry mechanism*

A.1 CCR5 inhibition

Development of anti-HIV genes against chemokine receptor CCR5 has become a main focus in anti-HIV HSPC gene therapy research CCR5 serves as a major co-receptor for HIV. After HIV binds to the CD4, the primary receptor, subsequent binding to CCR5 is essential for a successful HIV infection. Blocking this early phase of HIV infection can be highly effective in protecting the cells from CCR5 tropic HIV infection before HIV integrates into host genome for establishing stable infection.

A.1.1 Ribozymes mediated CCR5 inhibition

Ribozymes are catalytic RNA molecules with enzymatic functions, capable of cleaving their target RNA

A.1.2 RNA interference mediated CCR5 knock down

RNA interference (RNAi) is a powerful technology that relies on a small double strand RNA to trigger sequence dependent mRNA degradation through the cellular RNA Induced Silencing Complex (RISC)

A.1.3 Genome editing technologies for CCR5 knock out

Recently, genome editing strategies have been utilized to knockout CCR5 expression. Zinc Finger Nucleases, TALEN, mega-nuclease, PNAs (Peptide Nucleic Acids), and the more recently discovered CRISPR/CAS9 genome editing technology are capable of introducing insertion and deletion mutations (indels) in CCR5 gene in human cells to confer HIV resistance.

A.1.3.1 The Zinc Finger Nucleases (ZFN) mediated CCR5 knock out

The Zinc Finger Nuclease (ZFN) consists of a zinc finger DNA binding domain fused to a DNA cleavage domain from the FokI restriction endonuclease. CCR5 directed ZFN induces nucleotide indels in three steps: First, the CCR5 Zinc Finger domain binds its target site, then FokI cleaves the genomic site, and finally the mutagenic Non Homologous End Joining (NHEJ) system repairs the cleavage with indels.

A.1.3.2 The ZFN mediated CCR5 knock out and transgene knock in

CCR5 ZFN has a potential to be utilized to knock-in an anti-HIV gene into the CCR5 gene locus by the ZFN induced DNA double strand break (DSB) and cellular Direct Homologous Recombination (DHR) reaction. DHR is a DSB

induced cellular mechanism that uses homologous nucleotide sequences as a template to repair the damaged DNA. Therefore, it is possible to take advantage of this system by providing a transgene DNA template containing a sequence homologous to the target as well as a sequence of interest (mutation, reporter gene, therapeutic gene, *etc*). This "knock-in" approach provides the opportunity to integrate anti-HIV genes into a defined locus

A.1.3.3 TALEN-mediated CCR5 knock out

Transcription Activator-Like Effector Nucleases (TALENs) consist of DNA binding domains of transcription activator-like effectors from *Xanthomonas* that have been fused to the non-specific Fok1 nuclease. Like ZFNs, TALENs can be designed to induce sequence specific genome cleavages.

A.1.3.4 CRISPR/CAS9 mediated CCR5 knock out

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) technology has been utilized to knock-out the CCR5 gene. CRISPR/Cas9 is derived from the type II CRISPR-Cas9 bacterial adaptive immune system. The Cas9 protein introduces DSBs into a DNA sequence defined by a guide RNA (gRNA). The customization of the gRNA (typically around 20 nucleotides) enables the Cas9 to cleave a target gene in a site-specific manner, triggering a DNA repair system, such as the NHEJ system and/or the DHR in cases where both chromosomes have already been damaged.

A.1.4 CXCR4 inhibition

In anti-HIV gene therapy, while CCR5 has become a primary target to inhibit HIV entry, it is possible that HIV may escape from CCR5 inhibition and a CCR5 directed mono therapy may fail by emergence of X4 tropic HIVs. X4 tropic HIVs uses CXCR4 chemokine receptor for a co-receptor for entry. A recent clinical case resulted in the rapid emergence of CXCR4 tropic HIV in a patient who had been transplanted with stem cells from a Δ32/Δ32 homozygous CCR5 defective HSPC donor, highlighting the fact that viral escape mutations and pre-existing X4 tropic HIVs may be a major limitation to the CCR5 inhibition strategies [10]. Therefore, CXCR4 is another potential target for anti-HIV gene therapy. In 2003, Anderson et al. targeted CXCR4 using shRNA and protected cells against X4 tropic HIV strain, NL4-3. Genome editing technologies have also been applied to CXCR4 gene to achieve resistance to HIV infection.

A.1.5 A HIV fusion inhibitor

Another extensively studied an entry step anti-HIV gene is C46. C46 is an HIV fusion inhibitor. It was developed from the C-terminal heptad repeat of HIV glycoprotein (gp) 41 as an anti-HIV gene. It is analogous to the FDA approved soluble peptide drug Enfuvirtide (T20, Fuzeon). C46 has a membrane-anchored domain to stably express on cell surface as an anti-HIV gene product. It interacts with the N-terminal hydrophobic α-helix of HIVgp41, and therefore, interferes with the six-helix bundle formation of HIV and subsequent HIV and cell membrane fusion, all the while rendering cells resistant to HIV. C46 is highly potent for inhibition of both R5 tropic and X4 tropic HIV strains *in vitro* and *in vivo* humanized mice . It has been tested for anti-HIV HSPC based gene therapy experiments in non-human primates

B. Host restriction factors derived anti-HIV genes

Research efforts have identified a growing number of host restriction factors that can restrict HIV infection. Host restriction factors serve as endogenous antiviral systems in host cells. They are usually expressed constitutively at low levels and up-regulated upon viral infection through interferon response. They target key components of the virions, and provide a first line of defense against viruses

B.1 Human TRIMCyp / Mutated human TRIM5α

Rhesus macaque TRIM5α and owl monkey TRIMCyp have been identified as potent HIV restriction factors. TRIM5α is a protein ubiquitously expressed that can bind viral proteins, lead to their ubiquitination, and therefore, can direct them to the proteasome degradation pathway. The human TRIM5α does not inhibit HIV, and only mildly inhibits HIV-2, while rhesus macaque TRIM5α efficiently inhibits HIV, but not SIV. Although these xenogeneic host restriction factors can inhibit HIV, they are not suitable for clinical application because of their potential immunogenicity.

C. 2LTRZFP to inhibit HIV integration

HIV integrates into host DNA genome to stably reside in infected cells as a provirus. Inhibition of HIV infection prior to this step is critical to prevent establishment of chronic HIV infection. Sakkhachornphop et al demonstrated that 2LTRZFP-GFP, an anti-HIV zinc finger protein (ZFP) fused to GFP moiety, targets and binds to the 2-long terminal repeat (2-LTR) in the pre-integration complex, prior to genome integration, with nanomolar affinity.They also showed that there was more than 100-fold decrease in HIV capsid p24 protein production in HIV challenged SupT1 cells due to 2LTRZFP-GFP expression, suggesting that the

2LTRZFN-GFP transgene interferes with the integration activity of HIV.

D. Anti-HIV genes to inhibit later steps of HIV life cycle

CCR5 or CXCR4 gene inhibitors, C46, huTRIMCyp/TRIM5α and 2LTRZFP-GFP are designed to inhibit HIV and to protect cells from stable HIV integration into host cell genome. Although halting HIV infection at early steps before integration is important, complete reliance on early step inhibitors may not be enough to achieve stable control of HIV disease, since early step inhibitors may not be 100% effective as cells that still become infected will produce HIV. Development of anti HIV genes capable of inhibiting HIV at later steps after integration into host cell genome is important to inhibit HIV that are able to get around early inhibition.

D. Anti-HIV genes to inhibit HIV gene expression

D.1 HIV tat and transactivation response element (TAR) inhibitors

HIV Tat is an HIV transcriptional activator. Tat interacts with the transactivation response element (TAR) in HIV RNA transcripts and promotes the initiation of the viral gene expression and the elongation of HIV transcripts. Lee *et al.*developed a small interfering RNA directed to *tat* to inhibit HIV transcription and demonstrated HIV inhibition in *tat* shRNA transduced macrophages.

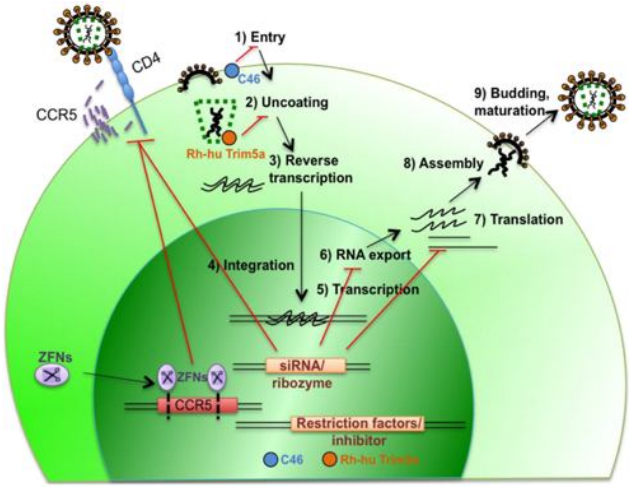

Figure 7 Stem cells based methods

VII. BROADLY NEUTRALIZING ANTIBODIES (bNAbs) FOR HIV ERADICATION

The recent discovery of highly potent, broadly neutralizing, HIV-specific monoclonal antibodies (bNAbs) provides a novel class of potential therapeutic agents. It has long been known that neutralizing antibodies can target the HIV envelope (Env) and effectively suppress viral replication in vitro. Until recently, however, bNAbs were few in number, targeted a narrow spectrum of HIV strains, and were not potent enough for practical use.

Over the last 5 years, the field has changed dramatically: new developments in high throughput single-cell BCR amplification and novel soluble Env selection tools have led to the isolation of new monoclonal antibodies with substantially increased potency and breadth. Phase 1 clinical trials of two CD4 binding site-specific bNAbs, VRC01 and 3BNC117, have shown that these antibodies are well tolerated in HIV-infected and HIV-uninfected adults, and 3BNC117 has been shown to provide antiviral activity in humans. Moreover, preclinical data in the non-human primate model using the V3 glycan-dependent bNAb PGT121 demonstrated reduction of proviral DNA in both blood and tissues. As a result, several laboratories are exploring the possibility that bNAbs may contribute to HIV eradication strategies.

A. EARLY EFFORTS UTILIZING ANTIBODIES TO TREAT HIV

A.1 Passive Immunotherapy with Pooled Plasma from HIV-Infected Donors

Passive transfer of anti-HIV antibodies has been tested for the treatment of HIV since the late 1980s, when investigators attempted to suppress viral replication with infusions of inactivated hyperimmune plasma pooled from HIV-infected donors. Jackson et al. (1988) demonstrated in six subjects that infusions of plasma from donors with high titers of anti-p24 (HIV core antigen) led to clearance of p24 antigen in the blood for up to 11 weeks. Karpas et al. (1988) showed similar results in 10 subjects following infusion of hyperimmune plasma. However, follow-up studies that were randomized and placebo-controlled were less clear in their findings.

A.2 Passive Immunotherapy with First-Generation Broadly Neutralizing Antibodies

Immunotherapy with anti-HIV antibodies changed in focus in the late 1990s, when the first bNAbs became available for clinical testing. Most clinical trials focused on the three monoclonal antibodies 2G12, 2F5, and 4E10. 2F5 recognizes the ELDKWA motif on the ectodomain of gp41; 2G12 is directed against N-linked glycans in the C2, C3, V4, and C4 domains of gp120; and 4E10 binds to a linear epitope (NWFDIT) that is adjacent to the 2F5 binding site on gp41.

Armbruster et al. (2002, 2004) showed in two studies that combinations of these antibodies were safe and well tolerated in 15 HIV-infected subjects at doses of up to 14 g of antibody over a 4-week period, and follow-up analyses by Stiegler at al. (2002) demonstrated that combination of these bNAbs led to reductions in plasma HIV RNA of up to 1.46 log10 copies/ml in a subset of volunteers. However viral escape with resistance to 2G12 was observed in 7/8 of subjects, particularly with mutations at N-linked glycosylation sites.

B. CURRENT EFFORTS TO DEVOLP ANTIBODIES FOR HIV ERADICATION

In the last 5 years, there has been an explosion in the number of potent HIV-specific bNAbs, several of which are being explored as therapeutic and prophylactic candidates. Advances in high throughput single-cell B cell receptor amplification, as well as new soluble trimeric Envs that can be used to select bNAbs, have led to the identification of multiple bNAbs with increasing potency and breadth. The bNAbs that are the most advanced in clinical development are outlined below.

B.1 CD4 Binding Site Antibodies

HIV entry into target cells is dependent on viral attachment to the CD4 receptor and is mediated through binding to a conformational epitope on the trimeric Env glycoprotein termed the CD4 binding site (CD4bs). Antibodies that are specific to the CD4bs can therefore block viral entry and inhibit viral replication. Moreover, the CD4bs is functionally conserved across diverse HIV strains, and thus anti-CD4bs antibodies can be extremely broad. Many CD4bs antibodies have now been isolated from human donors, and they share common structural features, such as heavy chain mimicry of the CD4 receptor.

One of the first CD4bs antibodies identified was VRC01, which was originally isolated from an HIV-infected individual that had been living with untreated infection for over 15 years. Like other CD4bs antibodies, VRC01 is highly somatically mutated, having evolved in response to continual virus escape over many years. Using a panel of 190 Env-pseudotyped viral strains representing all major clades and circulating recombinants, Wu et al. (2010) demonstrated that VRC01 neutralized 91 % of pseudovirions at a half maximal inhibitory concentration (IC50) of <50 μg/ml, and neutralized 72 % of primary isolates at an IC50 of <1 μg/ml. Preclinical challenge studies in non-human primates confirmed that VRC01 had protective activity in vivo as well.

In 2015, Ledgerwood et al. reported the results of the first clinical trial testing VRC01 in humans (VRC602). In this study, 28 healthy adults received varying doses of VRC01 ranging from 5 up to 40 mg/kg given as an intravenous infusion, as well as subcutaneously in some subjects at 5 mg/kg. Doses were given either once or twice, 28 days apart, and a subset of subjects received placebo. This study showed that among 43 administrations of VRC01 throughout the study, there were no serious adverse events, and local and systemic solicited reactions were mild. Subjects also did not develop anti-VRC01 antibodies, even though some possessed IgG1 GM allotypes that mismatched with VRC01. The pharmacokinetic analysis showed that VRC01 had a half-life of 15 days when given i.v. with 28-day trough levels that ranged from 35 to 89 μg/ml depending on the dose and frequency of administration. Based on preclinical data that had shown VRC01 was 50 % protective at concentrations of 18–28 μg/ml, the authors concluded that potentially protective VRC01 serum levels were observed in their subjects for up to 8 weeks following a second infusion. The potential utility of VRC01 in therapeutic and eradication strategies is currently being explored in HIV-infected subjects on and off antiretroviral treatment, and a phase 2b efficacy study to evaluate VRC01 for prevention of acquisition of HIV is expected to start soon.

B.2 V3 Glycan-Dependent Antibodies

PGT121 is a monoclonal antibody isolated in 2011 from an African donor that targets a V3 glycan-dependent site on the HIV envelope. PGT121 is distinct from other V3-specific monoclonal antibodies, like KD-247, because it does not bind simply to the GPGR region of V3. Instead, PGT121 has a long heavy chain complementarity determining region (CDR) that forms an antibody binding site with two functional surfaces. Structural studies suggest that PGT121 inhibits CD4 binding to gp120 despite the fact that it does not engage the classically described CD4 binding site. Instead, PGT121 likely interferes with Env receptor engagement by an allosteric mechanism in which key structural elements, such as the V3 base, the N332 oligomannose glycan, and surrounding glycans, including a putative V1/V2 glycan, are locked into a conformation that obstructs CD4 binding. By interfering with a highly conserved function required for viral entry, PGT121 has excellent potency and breadth of activity. For example, Walker et al. (2011) demonstrated that PGT121 had a median IC50 of 0.03 μg/ml when tested against a panel of 162 HIV pseudoviruses; this potency was a log higher than that observed for the CD4-binding site antibody VRC01 (0.32 μg/ml). In addition, Walker et al. demonstrated that PGT121 was able to neutralize 70 % of pseudoviruses tested.

The studies demonstrated that PGT121 exhibited antiviral activity in viremic, SHIV-infected rhesus monkeys, but these data did not directly show targeting of the viral reservoir. To address this question, ongoing studies are evaluating PGT121 in ART-suppressed, SHIV-infected rhesus monkeys. PGT121 is also being advanced into clinical trials, with plans for first-in-human, phase 1 testing in HIV-uninfected and HIV-infected adults in 2016. 10-1074 is a related V3 glycan-dependent antibody that is also undergoing phase 1 testing.

B.3 V2 Glycan-Dependent Antibodies

CAP256 is a recently described bNAb isolated from an HIV-infected individual in South Africa who had been infected (and subsequently superinfected) with HIV-1 subtype C for approximately 5 years before initiating antiretroviral therapy. Moore et al. (2011) demonstrated that CAP256 had a bias towards neutralizing subtype C and A virus, in some cases with remarkable potency. Epitope mapping studies showed that CAP256 bound a quaternary epitope found on the trimeric form of the envelope glycoprotein that was dependent on residues 159 to 171 in the V2 loop. Analysis of similar antibodies in this class revealed that the long heavy-chain CDR region 3 loops pierce the HIV-1 glycan shield to bind to the quaternary epitope at the apex of the HIV-1 spike where the V1V2 regions of two gp120 protomers meet. Reh et al. (2015) demonstrated that two CAP256 variants (VRC26.08 and VRC26.09) were also able to inhibit cell-cell virus spread.PGDM1400 was recently identified by Sok et al. (2014) by using trimeric HIV-1 envelope (BG505 SOSIP.664) as bait to select memory B cells from an HIV-infected donor by single-cell sorting. PGDM1400 exhibited exceptional breadth and potency. For example, PGDM1400 neutralized 83 % of viruses of a cross-clade 106-virus panel, with a median IC_{50} of 0.003 μg/ml. Structural studies revealed that PGDM1400, like CAP256, binds to the trimer apex via a very long (34-residue) CDRH3 arm. Moreover, the combination of PGDM1400 + PGT121 neutralized 98 % of tested global viruses at a median IC_{50} of 0.007 μg/ml. These data suggest the potential of bNAb cocktails for global coverage.

VII.CONCLUSION AND DISCUSSION

An important gateway to both prevention and care is knowledge of HIV-1 status.The increasing number of infected people and the disproportionate burden of infection in resource-constrained settings creates a scientific imperative to ensure research is done for people and in settings who stand to benefit most. Recent advances in generation of designer site-specific nucleases, specifically the advent of the CRISPR/Cas9 systems, have the potential to revolutionize many studies on HIV-1 biology. Immediate applications of this technology include *in vitro* studies of required host genes or of innate restriction systems and development of genetically altered animal models for HIV-1 infection. Longer term it seems likely that the ease with which gene editing systems can be developed and tested will lead to safe and efficient production of gene edited terminally differentiated or hematopoietic stem cells that are resistant to HIV-1 infection for re-introduction into patients. Anti-HIV HSPC based gene therapy has emerged as a potentially powerful approach to develop as an HIV cure strategy since the first case of HIV cure achieved by HIV resistant bone marrow transplants. Numerous anti-HIV genes capable of inhibiting different steps of HIV infection have been developed with state-of-the-art technologies including RNAi, ZFN, TALEN, and CRISPR/Cas9. The concept of using antibodies against HIV to suppress viral replication has been around for many years, but early efforts using hyperimmune plasma from HIV-infected donors and bNAbs with low potency and narrow breadth were largely disappointing. The advent of multiple new bNAbs with substantially greater potency and breadth has led to greatly expanded possibilities. Preclinical studies have shown that certain bNAbs can reduce viral loads and also lead to reductions of proviral DNA, particularly when given with latency reversal agents. Most importantly, early clinical trials have shown that bNAbs are safe, well-tolerated, and have antiviral activity in HIV-infected subjects. Additional clinical trials are planned for the coming years.

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