HIV Treatment, Towards Functional Cure

Kai Qi Zong

Ashford School, Ashford TN24 8PB, United Kingdom.

zongk@ashpupil.co.uk.

Abstract

Human Immunodeficiency Virus continues to be a dire threat to humanity as it leads to progressive CD4$^+$ T cell loss and immunological dysfunction, exposing the host to increased risk of lethal infections and cancer, HIV infection also contributes to cardiovascular disease, dementia, renal and hepatic dysfunction. Structural and functional analyses uncovered how HIV virus interact with host cells and the virus life cycle include binding, fusion, reverse transcription, chromosome integration, replication, assembly, and budding. High genetic variability and rapid evolution of HIV-1 lead to its worldwide spread and development of drug resistance. Currently, a wide variety of different therapeutics are being developed, which include reverse transcriptase inhibitor, protease inhibitor, integrase inhibitor, entry inhibitor etc., Combination of these current treatment methods can disrupt the life cycle of the virus at different stages that make HIV infection clinically manageable but remains incurable. Only limited reports of allogeneic hematopoietic stem cell transplantation from ccr5Δ32 homozygous to an HIV-infected patient leads to long-term remission without antiretroviral therapy. Here we summarize current HIV treatments and novel therapeutic modalities including broad neutralizing antibodies, multi-specific antibodies, genetic editing approach that could potentially lead to a cure to HIV infection.

Keywords

List HIV, Pathophysiology, Life Cycle, Antiretroviral Therapy, Capsid Inhibitor, Broad Neutralising Antibodies, Multi-Specific Antibodies, Genome Editing, Vaccination.

1. Introduction

1.1 History

Human Immunodeficiency Virus (HIV) was discovered in 1981 initially as a rare form of cancer and pneumonia in young homosexual men. It was thus named Gay-related Immune Disease (GRID) at that point in time. The disease was not observed to be contagious, so it was believed that heterosexual relationships were protected against HIV. In 1983, it was discovered that women could be infected with HIV through heterosexual intercourse. The 4 'H's, Homosexuals, Haemophiliacs, Heroin addicts, and Haitians were believed to be the most at-risk group according to the CDC. [47] [49]

The virus was believed to originate from primates in the form of Simian Immunodeficiency Virus. The viral strain of HIV-1 is most similar to the strain of SIVcpz in *P. t. Troglodytes* in Southeast Cameroon and SIVgor in western lowland gorillas. SIV is non-pathogenic in its natural host. It is believed that the cross-species infection was caused by a hunter or bushmeat vendor either cut or bitten by an infected chimpanzee or gorilla or was exposed to their blood on an open wound. Although the exact origin of the virus in human host is based on speculation, it is currently impairing the lives of 37.9 million individuals worldwide. [1]

1.2 Pathophysiology of HIV/AIDS:

The virus is commonly transmitted via unprotected sexual activity, hypodermic needles, blood transfusions, and from mother to child through the exposure of the mother's infected blood. Upon entering the bloodstream, the HIV virus will attach its gp120 glycoprotein to the CD4 receptors. After infecting the CD4$^+$ cells, it replicates quickly, resulting in the death of CD4$^+$ cells. Without the necessary assistance of CD4$^+$ cells, the B cells and CD8$^+$ Cytotoxic T cells cannot be activated to

produce humoral or cell mediated immune responses. As the condition progresses through the infection, latent, $CD4^+$ cells will eventually lower to 200 cells/ml of blood, reaching the stage of AIDS, leaving the patient susceptible to certain forms of cancers and opportunistic infections. [48]

The first stage of the infection is the Acute HIV infection. Between 2-6 weeks after infection, HIV patients may experience flu-like illnesses with symptoms such as but not limited to fever, sore throats, body rashes, exhaustion, joint pains, swollen glands, muscle pains. There are approximately 7 million virus particles/ml of blood when the infection just began, which infect and kill $CD4^+$ T cells. In addition to this cause, $CD8^+$ T cells also deplete $CD4^+$ T cell population by inducing infected cells to undergo apoptosis. The $CD8^+$ T cells are thought to be main contributor to the decrease of HIV virus load. Interestingly, due to the expression of CCR5 coreceptor in the mucosal $CD4^+$ T cells in the small intestines, they deplete at a higher rate than the $CD4^+$ T cells in the bloodstream. But however quickly $CD8^+$ T cells respond to this threat, $CD4^+$ T cells are fated to continuously decline. [2]

The second stage of the infection is known as the Clinical Latency. During this period, although the virus is still active, it reproduces very slowly. Patients typically will not exhibit any significant symptoms during this stage. Depending on the antiviral drug therapy of the patients, the Clinical Latency stage can vary in time length. During this stage, the virus can still be transmitted. At the end of the stage, the $CD4^+$ T cells will decrease in number and the HIV viral load will increase, leading to the final stage of the infection: Acquired Immunodeficiency Syndrome (AIDS). [2]

AIDS is the most severe stage of the infection process. The viral load concentration is drastically higher than the $CD4^+$ T cells concentration. With a weakened immune system, the patients are likely to be infected with opportunistic illnesses such as but not limited to pneumonia, tuberculosis, and different forms of cancer. The common misconception is that the HIV virus is fatal, but the truth is that it is the opportunistic illnesses that kill the patient. [9]

During the stage of AIDS, the HIV virus is directly affecting the neuropsychiatric, head and neck, cardiovascular, pulmonary, gastrointestinal, renal, endocrine, musculoskeletal, haematitic, dermatologic systems with disorders such as but not limited to neuropathy, retinopathy, atherosclerosis, pulmonary hypertension, non-alcoholic fatty liver disease, impaired glucose and lipid metabolism, myopathy, anaemia, eosinophilic folliculitis respectively. Common complications in this stage includes but is not limited to chronic psychiatric disorders, gingivitis, endocarditis, chronic obstructive pulmonary disease, viral hepatitis, chlamydia, invasive fungi, bone marrow infiltration, and fungal dermatoses to the respective bodily systems in the aforementioned order excluding the musculoskeletal system. A number of antiretroviral treatment-related adverse effects also exists, which together with the severity of this illness, calls for a need of our treatment advancement and the creation of a cure. [3]

1.3 HIV Structure and Genome:

HIV is classified as a lentivirus, a subgroup within retroviruses. The virion is approximately 100nm in diameter. It contains a cone-shaped capsid holding 2 copies positive-sense single stranded RNA. The virus contains protease, integrase, and reverse transcriptase enzymes to aid with its infection and replication process. [13]

The HIV genome, encodes for 16 viral proteins with its 9, 749 bases, playing essential roles in the virus' life cycle, including but not limited to the matrix, capsid, nucleocapsid, p6, viral infectivity factor, gp (glycoprotein) 120, gp 41. While the long open reading frames code for the structural proteins, the smaller open reading frames code for the proteins that regulate the viral life cycle. The proteins encoded typically function in pairs, the phenomenon was thus known as HIV pairwise proteins interaction. For example, GP120 and GP41, located on the membranes of the proteins, interact together for the virus to enter the $CD4^+$ T cell. So far, 10 interactions have been identified with their biological functions recorded. The pairwise interaction between more of these HIV proteins is a potential area for future studies and disrupting one mechanism could be a new potential treatment method. [8]

Two main types of HIV exist: HIV-1 and HIV-2. HIV-1 is currently more of the threat to our world as it is not only is it more infectious, it occurs all over the world while HIV-2 is mainly isolated to West Africa. The HIV-1 genome codes for viral protein U while the HIV-2 genome codes for the viral protein X. While the Vpx packaging is very much dependent on the leucine rich motif of p6Gag, Vpu is not packaged into virions. The difference in these mechanisms ultimately resulted in different behaviours of the 2 viruses. Due to the fact that most of the research undergone regarding HIV is to do with HIV-1, this paper will be mainly addressing HIV-1. [8]

1.4 HIV Life Cycle

The HIV life cycle consists of the steps: entry; uncoating; reverse transcription; nuclear import and integration; assembly, budding, and maturation; and reinfection. The virus undergoes this continuous cycle to quickly multiply and destroy the host immune system. [52]

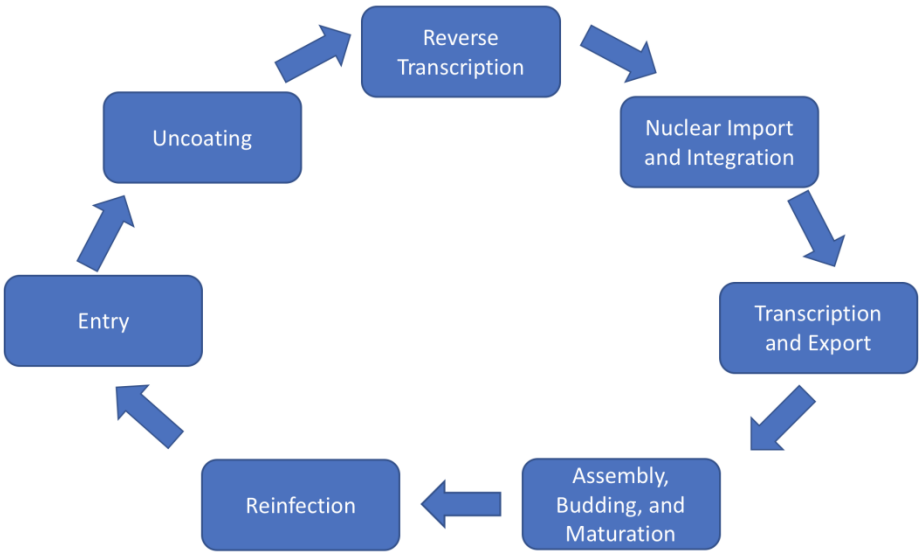

Figure 1. HIV life cycle

1.4.1 Entry

The CD4 receptor on $CD4^+$ T lymphocytes, macrophages, monocytes, etc. will interact with the gp120 on the membrane of the HIV cells. This induces a conformational change allows for the formation of a bridging sheet between the inner and outer domain of the gp120, allowing it to form an attachment with chemokine coreceptor CCR5 or CXCR4. The tips of gp41 form an attachment with the host membrane, it then changes its shape, bringing the membrane of the virus and the host together, allowing it to fuse together. [8]

1.4.2 Uncoating

In order for reverse transcription to occur, the capsid protein shell must be partially dissolved. N-terminal domains and C-terminal domains form ring-like structures, containing several promoters, can further assemble into fullerene cone that is the capsid protein shell. The initiation of the uncoating process may be further by the high concentrations of penton protein declinations near the narrow end of the cone-shaped shell. [13]

1.4.3 Reverse Transcription

There remains a limit on our understanding of this process, and whether this process occurs before or after nuclear entry still remains unclear. The enzyme responsible for the reverse transcription process is the reverse transcriptase (RT). RT is composed of the p66 and p51 protein subunits. Two functional active sites are present on the p66 subunit to undergo the process. The positive-sense single stranded

RNA is removed from the attached viral proteins by the RT and a complimentary DNA molecule is encoded. Due to the fact that the process is extremely error prone, mutations can often occur, resulting in the virus developing drug resistance. The RT is also able to act as a ribonuclease to degrade the viral RNA after its reverse transcription process, allowing complementary strands of the newly made viral DNA to come together to form the pre-integration complex (PIC). [8]

1.4.4 Nuclear Import and Integration

Due to the fact that PIC too large to pass through the nuclear pore complex, it is believed that the transportation process is active and dependent on viral and cellular components. Through experimentation, we found that mutated capsids lead to poor viral replication. This supports the notion that the capsid protein interacts with nucleoporin 358 to dock the virus on the Nuclear pore complex (NPC), with uncoating and reverse transcription ultimately occurring at the nuclear pore. The matrix on the other hand, does not seem to be essential, as HIV-1 is seen to have the ability to infect macrophages without a functional matrix. [18]

Viral protein R (Vpr), typically used to arrest cell cycles, was seen to dock the PIC to the nuclear pore complex. Due to the fact that Vpr contains nuclear localisation signals, it is able to enter the nucleus through its interaction with importin-α. Its role with the nuclear importation of HIV-1 is still, however, unclear. [18]

A number of host cell mechanisms also promote the nuclear importation of HIV, such as the short interfering RNA molecule that is able to help deactivate factor blocks inhibiting the entry of HIV, such as transportin 3, NUP153, and RANBP2. However, the exact mechanisms remain unclear. [18]

The enzyme involved in the integration of the PIC is called the integrase. It is responsible for 3' processing as well as DNA strand transfer. Intasome, or integrase-viral DNA nucleoprotein complex, is formed from integrase and PIC. The integrase uses the hydroxyl groups from the 3' portions to bind the intasome from the 5' phosphates of the chromosomal DNA. Host enzymes then complete the joining by repairing the gaps within the single strands of the unjointed 5' virus DNA. [16]

1.4.5 Transcription and Export

The transcription process is initiated at the U3 promoter within the upstream long terminal repeating circle (LTR). The viral transactivator of transcription efficiently elongates the LTR to allow for transcription for mRNA. The shorter mRNA can be exported out of the nucleus while the unspliced and singly spliced mRNA are bound together by the regulator of expression of virion proteins response element to the nuclear export factor CRM1. [18]

1.4.6 Assembly, Budding, and Maturation

During the last phase of the HIV life cycle, the membrane proteins of HIV, gp 120 and gp 41, are made from the originally coded gp 160. Gp 160 is assembled in the rough endoplasmic reticulum of the host cell, and is then transported to the Golgi apparatus, where the it is cleaved to form gp 120 and gp 41. The two proteins will move to the infected cell membrane where gp 41 will anchor the gp 120 onto the membrane. The group specific antigen p55 and the Gag-Pol precursor p160 attach on the inner surface of the membrane to begin forming the new virion and bud out of the infected cell. The new group specific antigen polyproteins are cleaved into the matrix, capsid, and nucleocapsid proteins with the viral protease. [13]

1.4.7 Reinfection

The newly formed virions can infect other cells with CD4 receptors within the blood plasma or the extracellular fluid. HIV can also spread from cell to cell directly through a virologic synapses. [12]

2. Current HIV Treatment Regimen

Antiretroviral Therapy (ART) is the treatment regimen recommended for all HIV patients, which they are recommended to begin as soon as they are diagnosed with the virus. ART is typically a combination of multiple medications from at least 2 different HIV drug classes. Individual ART drugs target and inhibit the different individual stages of the HIV virus replication. [19] [50]

2.1 Antiretroviral Therapy

2.1.1 Nucleoside Reverse Transcriptase Inhibitor

Nucleoside Reverse Transcriptase Inhibitor (NRTIs), like the name suggests, inhibits the reverse transcription step of the virus life cycle, and were the first type of drugs to be used against the HIV virus. Drugs to prevent this mechanism was manufactured and approved by the FDA as early as 1987 such as the Retrovir, containing zidovudine and azidothymine, and as recent as Epizom, containing abacavir and lamivudine. [26]

The inhibitors are converted to diphosphate or triphosphate metabolite forms in order to function. The metabolite essentially causes chain termination in the viral HIV after it incorporates itself into the chain as a nucleotide. [26]

There are different metabolic pathways to form pyridine and purine analogues. An example of a Pyridine Metabolic Pathway can begin from the Zidovudine (AZT) and Stavudine (d4T) to form a thymine analogue. Through a series of enzymatic reactions, AZT and d4T form AZT-TP and d4T-TP respectively, which ultimately come together to form deoxythymidine triphosphate molecule (dTTP), the thymine analogue that can inhibit the reverse transcription. Lamivudine (3TC) and Emtricitabine (FTC) go through a similar series of reactions to ultimately form deoxycytidine triphosphate (dCTP). [26]. Purines analogues deoxyadenosine triphosphate (dATP) and deoxyguanosine-triphosphate (dGTP) are ultimately formed from a series of complicated enzymatic activities from Tenofovir disoproxil fumarate (TDF), Tenofovir alafenamide (TAF), and Didanosine (ddI) for dATP and Abacavir (ABC) for dGTP. [26]

2.1.2 Nonnucleoside Reverse Transcriptase Inhibitors

Unlike NRTIs, NNRTIs bind to the reverse transcriptase enzyme itself in order to inhibit the reverse transcription of the virus life cycle. Certain inhibitors were manufactured and approved by the FDA and approved by the FDA since 1996 like Viramune, which contains nevirapine and NVP, while advancements, such as Viramune XR, manufactured and approved by the FDA and approved by the FDA in 2011, will not be released immediately. [25]

The discovery of 4,5,6,7-tetrahydroimidazo[4,5,1-jk][1,4]benzodiazepin-2(1H)-one and -thione (TIBO) compounds led the beginning of research into NNRTIs. Through careful experimentation, it was observed that RT activity was inhibited after time of addition, it was only active on HIV-1 RT, and that it had a template-primer dependency. It was thus concluded that the effectiveness and specificity of TIBO made it an effective example of NNRTIs. [25]

2.1.3 Protease Inhibitors

Proteases are vital for the HIV virus to produce functioning viral enzymes and structural proteins as it hydrolyses the Gag and Gag-Pol polyproteins to form these protein molecules. Protease Inihibitors (PIs) have been manufactured and approved by the FDA since 1995, with Invirase, containing saquinavir mesylate (SQV). More recently FDA approved PIs include Prezita, containing Darunavir, which was manufactured and approved by the FDA since 2006. [28]

The first generation of PI were based on hydroxylethylamine and hydroxylethylene isosteres, using its central hydroxyl group to mimic the intermediate of the hydrolysis process by binding to the catalytic aspartic acid residues on the PI. But due to the peptidic nature of the PIs, it results in high metabolic clearance, low half-life, and poor oral bioavailability, it required frequent dosing. In addition, multiple side effects such as gastrointestinal distress and nausea were observed. Furthermore, mutations resulting in drug resistance became a growing problem as the residues in the active sites began to mutate while secondary mutations made up for the detriments caused by the primary mutations. Ultimately, a new generation of PI was needed to effectively treat HIV. [28]

Second Generation PIs such as darunavir were developed to help improve upon the drawbacks of the previous generation. Darunavir is potent and also inhibits the essential dimerization process of HIV protease, as this process gives the enzyme its proteolytic properties. This dual inhibitory property has been fairly effective in countering the drug-resistance mutations of HIV. [28]

2.1.4 Fusion Inhibitors

Fusion inhibitors, as the name suggests, inhibits the mechanism of membrane fusion between the HIV virus and host cell during the virus life cycle, preventing it from entering. The envelope glycoprotein complex, comprised of gp120 and gp41, allows for the attachment and fusion process. Fuzeon, composed of enfuvirtide, competitively binds on the gp41 and prevents it from undergoing conformational changes that leads to viral entry. [24]

2.1.5 Entry Inhibitors

Unlike fusion inhibitors, entry inhibitors are CCR5 or CXCR4 coreceptor antagonist on the immune cells to prevent viral entry of the replication process rather than inhibitors of viral envelope glycoprotein complex. The antagonists are categorised based on size, with large molecules such as PRO-140, medium molecules such as Met-RANTES, and small molecules such as maraviroc. Most CCR5 antagonists fall in the small category, they essentially mimic chemokines, the coreceptor's natural ligand, inhibiting their attachment for viral entry. [20] [21]

2.1.6 Integrase Inhibitors

Integrase Strand Transfer Inhibitors (INSTIs) inhibits the integration process of the virus life cycle. Drugs classified as INSTIs include Isentress, Tivicay, and Viteka. The inhibition functions by binding the interface of macromolecular components, such as the intasome, forming conformational intermediate, interfering with its dynamic activity, and preventing it from being incorporated into the host DNA. [22]

2.2 Evaluation of ART

Antiretroviral therapy utilises multiple inhibitory mechanisms to hinder the life cycle of HIV viruses, effectively preventing further progression in the infection progress. Although the treatment efficacy is good and is fairly easy to administer, drawbacks still exist. The treatment requires strong patient adherence and missing one tablet could mean disease progression. In addition, although ART targets multiple stages of the HIV life cycle, a mutation in the virus could mean a development of drug-resistance in at least one of the stages, which also ultimately allows the virus to progress. Thus, there is still a great need for a cure for HIV.

3. Novel Therapeutic Approaches in HIV Treatment

Numerous research is being done attempting to tackle the HIV virus at countless different angles. Further inhibition combatting against drug-resistance development are being studied. In order to cure an individual of HIV, both infected cells that actively produce new virus and the 'HIV reservoir' that are infected with HIV but have not produced new HIV (latent stage of infection) need to be cleared or the HIV virome that integrated in the host genome need to be inactivated . In order to tackle those situation, novel capsid inhibitors, antibodies and vaccines that could trigger immune response to eliminate virus, and genome editing technology are being extensively studied.

3.1 Capsid Inhibitor

Compounds that bind to the capsid protein has been found to prevent infection steps such as reverse transcription and nuclear entry, while others disturb capsid assemble and lead to non-infectious progeny virions. Distinct binding sites are present for different inhibitors on the CA N-terminal domain (NTD) and C-terminal domain (CTD). [30]

Before the discovery of inhibitor GS-CA1 in 2017, no direct link has been established between capsid inhibitors and their weak potency has not allowed for further clinical trials. Fortunately, GS-CA1 was highly potent; it is able to bind to the NTD-CTD intersubunit interface while reducing particle infectivity and inhibiting infection from mature viral capsid. [30]

GS-CA1 has been observed to be both more potent and selective than EFV of NNRTIs, DTG of INSTIs, and ATV of PIs at inhibiting HIV-1 in the peripheral blood mononuclear cells (PBMC), with an EC50 value of 130±80 pM. In addition, it has also been effective against both Wild Type (WT)

and drug-resistant HIV viruses. In comparison with older capsid inhibitors, GS-CA1 has a much higher potency and lower cytotoxicity. [30]

In seven cases of mutations on the CA (L56, N57, M66, Q67, K70, N74, and T107), while all other functions are not hindered, GS-CA1 was observed to be ineffective while antiretroviral drugs were effective. In separate case, GS-CA1 selected resistance-associated mutations (RAMs) were introduced to a replication-competent reporter HIV-1. It was found that replication capacities for the most common GS-CA1-selected RAMs were compromised in $CD4^+$ T cells. [30]

3.2 Broad Neutralising Antibodies

HIV broadly neutralising antibodies (bNAbs) are originally from HIV patients with a high level of anti-HIV neutralising activities. These antibodies target the gp120 on the membranes of HIV viruses as an immune response. Several bNABs are being clinically developed, such as VRC01, 3BNC117, and 10-1074. [34]

Clinical trials have indicated that bNABs were not only well tolerated, but also linked with virologic activity and enhanced immune function. Through a two-amino acid substitution, a crystallisable fragment domain is introduced, increasing the half-life of the serum of bNABs, allowing for infrequent dosing. Current studies are focussing on combining various bNABs and antiretroviral drugs. [34]

3.2.1 Ibalizumab

The humanised IgG4 antibody, Ibalizumab, inhibits HIV entry allosterically from the CD4 receptor. Due to its distinctness from the major histocompatibility complexes on the CD4 receptor, Ibalizumab is less likely to reduce immunosuppression. It is currently recommended for patients with HIV that are resistant to different types of drugs and are failing their current therapies. [23]

Ibalizumab is administered intravenously with a dose of 2000mg, with additional 800mg biweekly. Although antiviral activity was observed, frequent cases of rebound of viremia was observed, even cases of resistance. [23]

Phase III clinical trials for ibalizumab, which involve standard treatment as control, 31 patients with a mean baseline viral load of 4.5 log10 copies per millilitre and CD4 count of 150 per microlitre were tested. 25 weeks after the control period, patients with Ibalizumab with an optimised background regimen had a mean decrease of 1.6 log10 copies per millilitre from the baseline values. Approximately 43% of patients were observed to have a viral load less than 50 copies per millilitre and 50% of patients were observed to have a viral load less than 200 copies per millilitre. [40]

Out of the 10 patients who exhibited virologic failure or rebound, 9 had a lower degree of susceptibility to ibalizumab than the observed degree at the baseline value. 4 patients in the study unfortunately died from underlying diseases, but only 1 patient had a serious adverse effect of immune reconstruction inflammatory syndrome that was deemed related to ibalizumab. The most common adverse effect observed was diarrhea. [40]

It was ultimately found from the Phase III trials that against multiple drug resistant HIV-1, ibalizumab induced significant antiviral activity. [40]

3.2.2 PRO-140

R5-tropic HIV viruses are a strain of HIV that infects $CD4^+$ T cells by attaching to the CCR5 coreceptor. PRO-140 is a humanised monoclonal antibody which has the ability to bind to CCR5 coreceptor and inhibits the entry of R5-tropic HIV viruses. As it is distinct from CCR5 antagonist maraviroc binding site, PRO-140 is effective against maraviroc resistant viruses. With PRO-140 alone at a 5mg/kg dose intravenously or 324mg subcutaneously, exclusive R5 tropic patients viral load were rapidly suppressed. PRO-140 is being tested for in patients with CCR5 virus baseline as a weekly maintenance. The drug can currently only be effective on patients infected exclusively with R5-tropic HIV-1. PRO-140 was granted Orphan Drug status by the FDA, giving it a 7-year window of exclusive right to develop it while reducing its tax. Fortunately, the drug is progressing towards approval. [38]

3.3 Bi-Specific and Multi-Specific Antibodies

3.3.1 Bi-Specific Antibodies

Bi-specific antibodies (bsAbs) have the ability to bind to two antigen binding sites (Ag-bs). Early studies found that two pepsin cleaved IgG molecules can lead to formation of two Ag binding fragments (Fab), which can ultimately form a chimeric molecule with the ability to bind to two separate Ag-bs. Since then, the rational design of bsAbs has improved and is now applicable to cancer, autoimmune disease, and infectious diseases. [32] [33] [34]

In the battle against different strains of HIV, more and more bsAbs treatments were designed and tested. The major challenges of bsAbs based HIV treatment include envelope diversity, bNAb escaping mutations, and access to follicular areas in the central nervous system or lymph nodes where HIV can establish reservoirs. The CrossMab format of bsAbs has been tested to target more than one envelope element to overcome these obstacles. Certain combinations, such as VRC07 and glycan dependent PG9-16, have demonstrated both increased breadth and potency while targeting CD4 binding sites. [32]

Envelope binding can be improved by switching the hinge domain of IgG1 to the hinge domain of IgG3, as it offers greater flexibility due to its greater length and different amino acid composition. The engineered IgG3C-CrossMab, with two FAB arms targeting both CD4 binding sites and gp120 V3-glycan epitome, demonstrated increased efficiency in both neutralisation potency and breadth. [33]

The study assessed the IgG3C- hinge variants of PGT151/10-1074, 8ANC195/PGT128, 3BNC117/PGT135 biNAbs and their respective parent bNAbs for their in vitro neutralisation activity against an extended multiclade virus panel. It was found that the representative IC50/80 titres (μg/ml) of bNAbs 3BNC117, PGT135, 3BNC117/PGT135 bNAbs exhibited substantial increasing in their neutralisation activity in comparison with their parental bNAbs. [34]

In this study, the 3BNC117/PGT135 IgG3C – bNAb in vivo therapeutic activity was compared with the 3BNC117 and PGT135 bNAb mixture in HIV-1-infected humanised mice. It was found that viremia levels significantly decreased in the 3BNC117/PGT135 IgG3C – bNAb treated mice. [34]

3.3.2 Multi-Specific Antibodies

bNAbs targeting CD4bs, MPER, and V1V2 glycan sites were found to be linked in an alternative IgG configuration, allowing the inversion of the mode of the V regions, arranging it one after another, with each region able to interact with its own target. Trispecific antibodies are able to fully protect non-human primates against a mixture of different simian HIVs with a mucosal challenge, an improved breadth was observed from the parent bNAbs. [44]

The study measured the IC50 (half maximal inhibitory concentration) neutralising titres of VRC01, PGDM1400, and VRC01/10E8v4-PGDM1400 against the replication competent SHIV BaLP4 or 325c. All animals in the study were given 5mg/kg of antibodies intravenously. 5 days after administration of the antibodies, the plasma viral load in the animals were challenged with the mixture of SHIV BaLP4 and 325c. It was found that none of the macaques with trispecific antibodies were infected while most of animals treated with VRC01 and PGDM1400 alone were infected. [44]

3.3.3 Strategies for Designing Bi-specific and Multispecific Antibodies

Bi- and tri-specific antibodies with the same cross-link adjacent promoters could be generated when a structural base approach was developed while considering the topology of the complex bNAb-envelope trimer and the vitality of the inter-promoter distance. [33]

Alternatively, bsAbs that target CD4 and CCR5 on the host cells could be generated to inhibit virus entry. [33]

The HIV specific bsAbs could target the glycoprotein on the envelope of HIV viruses while simultaneously engaging the CD3 receptor on the $CD8^+$ Cytolytic T cells. Studies in the late 1990s began a strategy to generate T cells activating HIV bsAbs with anti-CD3 to retarget host effector B and T immune cells while using its second targeting arm to identify viral elements. [33]

In one study, Unstimulated $CD4^+$ T cells were infected with either HIV-1 viruses isolates BaL, IN, or RW cocultured with CD8+ T cells from the same individual in either the presence or absence of active Dual-Affinity Re-Targeting (DART) for HIV and CD3 mix at different concentrations between 0.6-2000 pM and at $CD8^+$ T cell to $CD4^+$ T cell ratio of 2:1 for 72 hours. No significant killing was mediated by 10E8 with CD3 or VRC01 with CD3 DARTs. Mixtures of DARTs vary in effectiveness against different HIV-1 isolates, but PGT121 with CD3 was observed to be significantly effective against all 3 isolates. [43]

In a separate study, unstimulated PBMCs from 4 different HIV patients on suppressive combined antiretroviral drug therapy were cultured with either 400pM a mixture of either of PGT121xCD3 and 7B2xCD3 or 400pM of Rous Sarcoma Virus. The supernatant HIV RNA was counted on the 8^{th} and 14^{th} day. And on the 14^{th} day, a significant reduction of HIV RNA was observed in 3 of the 4 patients treated with the HIVxCD3 DART combination, and no significant difference was found between control DART and no DART. [43]

3.4 Genome Editing Strategies against HIV

Although the current HIV ART is effective in blocking the virus replication, it is not able to target the HIV provirus in the cells that help form the viral reservoir. HIV patients have to undergo a lifetime of treatment because drug therapies will cause viral rebound. To prevent the need for a treatment duration this long, researchers are looking for a way to permanently deactivate the provirus, which may be a cure for HIV. Many studies have found that the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) can be used to attack the virus DNA. The focus of the paper will be on how the endonuclease activity can lead to the deactivation of the provirus, but also how to prevent the virus escaping. [36]

3.4.1 CRISPR-Cas9 System

The system was a derivative of the CRISPR-Cas system that essentially identifies and cuts nucleic acids from pathogens. The CRISPR-associated endonuclease Cas9 of Streptococcus pyogenes (spCas9) was made into a genome editing tool that cuts the double stranded DNA in eukaryotes. A 20-nucleotide sequence in the guide RNA (gRNA) directs Cas9 to the complimentary DNA target in order to mediate the sequence specificity. Only the complementary sequences flanked by protospacer adjacent motif (PAM) can be cut by the Cas9. The cellular DNA repair mechanisms, such as non-homologous ends joining (NHEJ), and microhomology-mediated ends joining (MMEJ), can then in turn fix the broken double stranded DNA from the Cas9 cleaving. [36]

3.4.1.1 Cas9/gRNA Attack of HIV DNA

The gRNA guides the Cas9 to cleave the HIV DNA 3 nucleotides away from the PAM. Through NHEJ and MMEJ, the double stranded DNA is repaired with different types of mutations, most of which deactivates the virus. However, in some instances, the mutations allow the virus to continue replicating while not being recognised by the gRNA, thus escaping from the Cas9. [36]

In one study, SupT1 cells transduced with Cas9 and gRNA expressing lentiviral vectors were infected with HIV LAI to monitor virus replication through measuring CA-p24 level in the culture supernatant as well as through virus-induced syncytia formation. Despite some gRNAs potency to suppress virus reproduction, it could only delay it briefly, allowing breakthrough virus replication to occur. In addition, the potency of the gRNA inhibition had no correlation with the amount of time viral replication could be delayed. Targeting the high conserved sequences proved to be more effective in sustaining antiviral activity than targeting less conserved regions. [29]

In another study, the single and dual gRNAs were tested in their effectiveness to silence HIV-1 DNA in 293T cells with plasmids expressing HIV-1 LAI, Cas9, and single or dual gRNAs. The CA-p24 levels were monitored in the culture supernatant 2 days after the transfection to calculate viral gene expression. Sup-T1 cells were transduced with lentiviral vectors expressing single or dual gRNAs as well as Cas9 were infected with the HIV LAI virus and cultured for 60 days. It was later observed that a high CA-p24 level was present for control gRNAs, a significantly reduced CA-p24 level for

single gRNAs, and a further reduced CA-p24 level for dual gRNAs. It could thus be concluded that dual gRNA was very effective against HIV reproduction. [35]

Attacking the HIV DNA at 2 different viral domains guided by 2 different gRNAs or with one gRNA targeting both the 5' and 3' region of the LTR can result in either excision or dual-site mutation. Simultaneous cleaving can result in excision of intervening fragment. Certain gRNA combinations, from targeting highly conserved essential sequences that effectively prevent viral replication, ultimately resulted in hypermutation, due to repeated Cas9 attack on point-mutated targets. In other words. using dual gRNA to guide the CRISPR-Cas9 could potentially be a cure for HIV. [36]

3.5 Vaccines

Although hundreds of vaccine candidates were tested for HIV-1 vaccines, only 6 HIV-1 vaccine efficacy trials were completed. Among the 6 trials, only RV144 study showed a significant reduction in HIV infection rates (31.2% protection). A large of amount of information was gathered, such as STEP and HVTN505 vaccinations enhanced viral evolution, which suggests that there was an immune pressure on the virus, and additional information that viral vector-based vaccines are more effective than multi-dose protein-based vaccines. The hope that HIV vaccines may be a viable cure is supported by the rich HIV vaccine clinical trials pipeline, a repeat of the RV144 trial, and the initiation of the HVTN702. [31]

3.5.1 RV144 Study

The "Thai Trial" gathered a total of 16402 healthy participants at heterosexual risk of HIV infection. The participants were divided into vaccine and placebo groups. The vaccine efficacy was found to be at 26.4% in the intent-to-treat analysis. The protocol was modified, however, due to the fact that seven participants were removed for being HIV positive since the beginning of the study. The vaccine efficacy thus rose to 31.2%. [41]

After the study, it was analysed and estimated that the vaccine efficacy was 60.5% in the first year. The efficacy declined to 31.2% after the first year. The protection was found to be due to the development of non-neutralising IgG against the V1/V2 region of HIV-1 instead of low-level induction of neutralising antibody. However, through sieve analysis, it was found that breakthrough viruses in the participants had vaccine-induced immune pressure within the V1/V2. [41]

3.5.2 Preclinical Evaluation of Novel Protein Based Immunogens

Vaccine development studies mainly focussed on creating subunit vaccines, which is advantageous regarding the potential safety profile over heat-killed and live-attenuated whole virus vaccines. Studies focussed on creating recombinant proteins of different variants of envelope glycoproteins gp120 and gp160 from different viral clades or strains and assessment of immunogenicity of different viral proteins from different gene expressions. Preclinical studies have tested the potential for countless combinations of vaccine regimen for recombinant proteins, expression vectors, delivery timeframes, and also methods of delivery. [31]

4. Conclusion

The HIV virus continues to be a threat to our world, and it can easily be one of the most complicated diseases we know of. It is fascinating how the virus is able to take control of the one thing in our body able to combat against it: our immune system. Currently, we can prevent the further progression of the virus through different drugs of the antiretroviral therapy by inhibiting the virus replication at its various stages, but at this point, we do not have a viable cure for all HIV patients. Although allogeneic hematopoietic stem cell transplantation from ccr5Δ32 homozygous has been successful in curing HIV in 2 different cases in the "Berlin" and "London" patients, it is viewed as a treatment for leukaemia. However, due to the complicated process of stem cell and bone marrow transplants as well as the difficulty of finding a viable match, it cannot be considered as a viable cure for HIV.

Researchers continue to develop potential cures from the different angles of capsid inhibition, broad-neutralising antibodies, bi- and multi-specific antibodies, genome editing, and vaccination. We are not yet at the finish line of curing HIV, but we can almost see it on the horizon.

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