

Running head: Human Immunodeficiency Virus: A Literary Review on Gene Editing, Cytotherapy, and Medicinal Marijuana as Therapies for HIV.

**Human Immunodeficiency Virus:
A Literary Review on Gene Editing, Cytotherapy, and Medicinal Marijuana as Therapies
for HIV.**

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ABSTRACT

Human immunodeficiency virus (HIV) affects about 40 million people world-wide; there is currently no cure for HIV or Acquired Immunodeficiency Syndrome (AIDS). This paper will begin by explaining viruses/retroviruses and HIV infection/progression at the molecular, cellular, and organismal level. Common drug therapy will be mentioned, followed by in-depth discussions on the processes of three different therapies that combat HIV-1 at either the molecular, cellular, or organismal level. Once the virus has been integrated in the genome, antiretroviral therapies (ART) begin. At the molecular level, gene silencing has the power to essentially “shut down” or “cut out” genes from the integrated proviral genome that therefore stop its reproduction and transmission to other cells. The machinery discussed will include CRISPR-CAS9, and short interfering RNA (siRNA). At the cellular level, cytotherapy is designed to transplant cells for replacement of damaged tissue and/or cells. The mechanisms for this will include stem cells, human embryonic stem cells (hESCs), umbilical cord blood stem cells (UCBs), and induced pluripotent stem cells (iPSCs). Symptoms of HIV at the organismal level are detrimental. Medical marijuana has recently been approved in dozens of states for HIV/AIDS. Medical marijuana has positive/beneficial effects on those who suffer with neuropathy, nausea, lack of appetite, diarrhea, undesired weight loss, depression and/or anxiety, and ultimately AIDS wasting syndrome from HIV and/or ART drugs. This paper will include not only a literary review analysis on the matter, but also an interview with someone HIV+ who uses marijuana as a therapy. This thesis will also include an outlook on the most promising therapies, HIV preventative medicine/vaccine research, as well as any disadvantages/challenges of the ones discussed.

1. INTRODUCTION ON VIRUSES AND RETROVIRUSES.

Viruses are composed of genetic material enclosed in a protein capsule. HIV, like many other viruses, contains an outer lipid envelope with glycoproteins (see figure 1). Viruses are not considered cells because they have no biosynthetic pathways or metabolism of their own, but depend entirely on the cells they infect to survive. After contact with a host cell, the virus will incorporate its genetic material into the host cell's genome and hijack its functions, generating viral genetic material and viral proteins instead of a normal cell's products. Retroviruses compose a large group of RNA viruses. They are identified by their unique structure, but mostly because of their replication strategy (Coffin, Hughes, and Varmus, 1997).

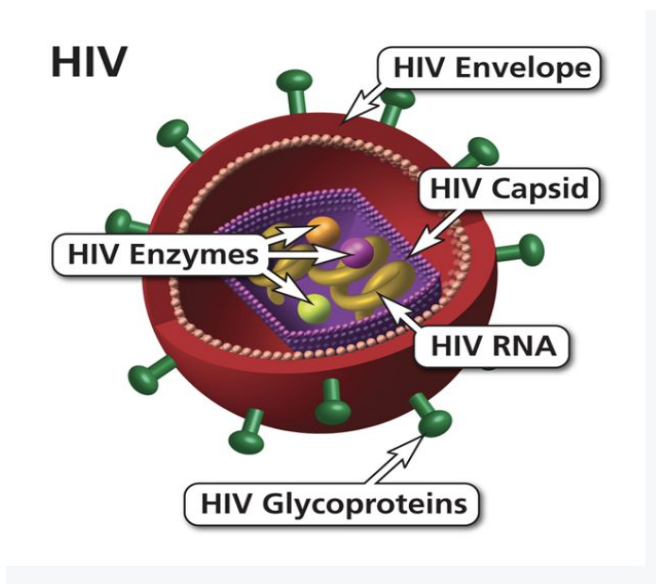


Figure 1- Structure of HIV

Source: Human Immunodeficiency Virus (HIV) Definition. (n.d).
Retrieved from
<https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/325/human-immunodeficiency-virus>.

The lipid envelope of retroviruses contain glycoproteins, proteins with oligosaccharide chains attached to the protein. These membrane markers are extremely important in their pathogenesis since the first step of viral infection is to identify a receptor on the host cell surface and for the virus to bind to it. In most cases, the first attachment site is a glycan (Banerjee and Mukhopadhyay, 2016).

A. THE CENTRAL DOGMA, RETHOUGHT.

The “Central Dogma” states that genetic material moves in one direction. DNA is transcribed to RNA, and RNA is translated to protein (Watson, 1965). The hallmark of retroviruses is their replication strategy, reverse transcription of the virion RNA into double-stranded DNA and the integration of this viral DNA into the host cell genome, due to a viral encoded polymerase that copies RNA into DNA (Coffin et. al, 1997) (see figure 2).

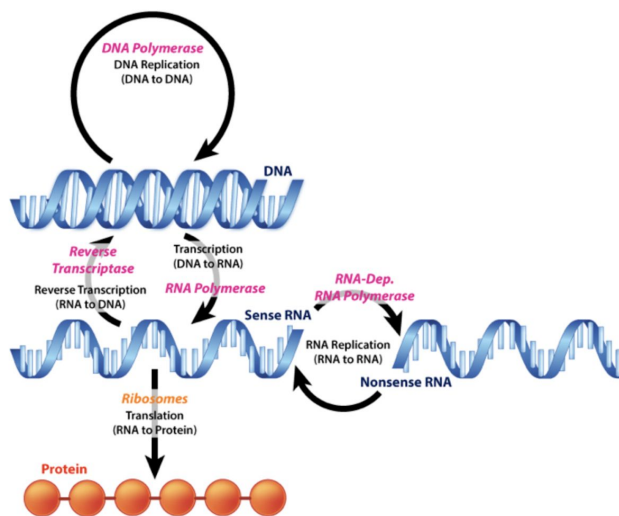


Figure 2- Modern Central Dogma

Source: Central Dogma. (n.d).
Retrieved from
<http://oregonstate.edu/instruct/bb451/451material/OutlineMaterials/34Translation.html>.

Viruses like HIV reverse the flow of genetic material, hence the term “retrovirus,” (see figure 2). Retroviruses can be simple or complex, all retroviruses code for 3 virion proteins. *Gag* codes for the virus matrix (p17), capsid, and nucleoprotein structures (p24, p7, p6). *Pol* codes for the reverse transcriptase and integrase enzymes. *Env* codes for the surface and membrane components of the protein coat (gp120, gp41). *Pro* codes for the virion protease. Complex viruses also code for additional regulatory sequences. *Tat* (named *tax* in figure 3), changes the affinity of the host cells RNA polymerase to the viral promoter (Klaus, 2003). It stimulates production of transcription of full-length RNA transcripts. Most RNA processing occurs in the

nucleus, fully spliced mRNA (exons) are transported into the cytoplasm for packaging. *Rev* (named *rex* in figure 3), exports unspliced or partly-spliced mRNA into the cytoplasm, and through nuclear localization signal (NLS) can target the resulting protein to the nucleus (2003).

HIV is a complex virus.

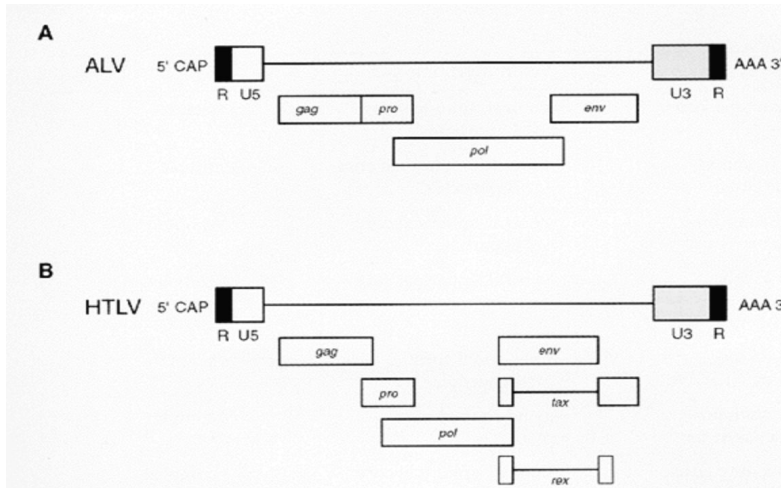


Figure 3- Genome of a (A) simple and (B) complex retrovirus reading frame.

Source: Coffin JM, Hughes SH, Varmus HE, editors. Retroviruses. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997. The Place of Retroviruses in Biology. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK19382/>

There are two strains of HIV, HIV-1 and HIV-2. HIV-2 is a less virulent infection compared to HIV-1. HIV-1 is responsible for most of the global AIDS pandemic (Campbell-Yesufu & Gandhi, 2011). Over 1,000 people contract HIV every day in South Africa. HIV-2 was initially found in West Africa, but has spread to Africa, Europe, and the United States. Prevalence in West Africa has dropped significantly in recent years, especially among the youth (Van der Loeff et al, 2006; Da Silva et al, 2008), but still affects about 2 million people. HIV-1 alone will be considered in this thesis.

2. INFECTION AT THE MOLECULAR LEVEL

HIV is transmitted through body fluids, such as blood or semen. HIV can also be transmitted from a mother who has HIV to a child, during pregnancy or breastfeeding. Once in the body, the HIV replication cycle begins. The lifecycle of viruses have 6 key steps:

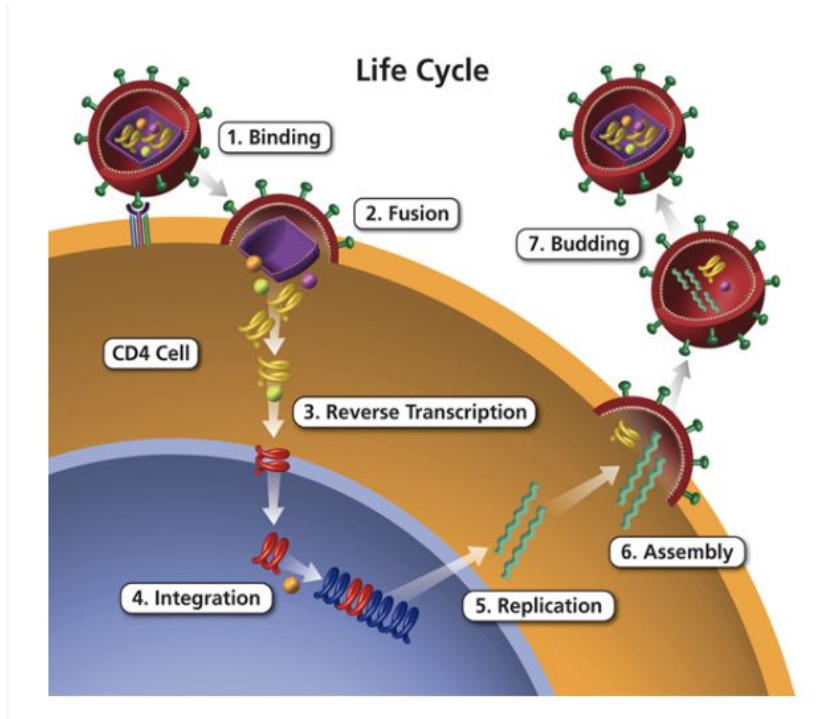


Figure 4- Life Cycle of HIV.

Source: Life Cycle

Definition. (n.d.).

Retrieved from

<https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/1596/life-cycle>.

A. BINDING

HIV glycoproteins gp120 and gp41 are essential for virus recognition (gp120) and entrance (gp41) into targeted cells (Fanales-Belasio, Raimondo, Suligo, and Butto, 2010). Gp120 binds with both a CD4 glycoprotein (expressed on most T helper cells (T-lymphocytes and T-cell precursors), monocytes/macrophages, dendritic cells, and microglial cells of the nervous system) and a chemokine receptor, small proteins that mediate the attraction of immune cells in an inflammatory response (2010). The chemokine receptor is either CXCR4 or CCR5. Depending on the chemokine receptor, HIV-1 either has CXCR4 tropism or CCR5 tropism. The

chemokine receptors on our T-lymphocytes and other cells mentioned are essentially locks which HIV has the key for. It's important to note that T cells (like B cells) are vital to the adaptive immune system, and depletion of immune cells is detrimental to an organism. The gp120 and gp41 complex changes shape after binding with a CD4 T cell. The shape change, as well as the double binding with a chemokine receptor elicits a humoral response, as would be with any viral invasion. However, in HIV infection the humoral response is known to be ineffective to stop viral propagation because the antibodies produced by most are non-neutralizing, meaning they recognize viral epitopes (part of the antigen that is recognized by an antibody) that fail to interfere with the replicative cycle of the virus (Carrillo et al., 2015).

B. FUSION

With a more stable double-attachment, gp41 and the chemokine receptor fuse with the cell membrane and allow for the entry of the viral capsid (Fanales-Belasio et. al, 2010). The virus then “uncoats” itself in the cytoplasm, freeing the RNA in the host cell.

C. REVERSE TRANSCRIPTION

Once in the cell, Reverse transcriptase uses the viral RNA to make a single stranded DNA (SSDNA) and a complementary DNA strand (cDNA) to make double stranded viral DNA (DSDNA). This conversion allows HIV to enter the CD4 cell's nucleus and combine with the host DNA. The rapid rate of HIV evolution is largely attributable to the error-prone nature of reverse transcriptase, which plays an important role in viral replication yet lacks proofreading activity (Andrews and Rowland-Jones, 2017).

D. INTEGRATION

HIV releases the enzyme integrase, which will bring the viral DSDNA into the nucleus and integrate the DSDNA into our own DNA as a “provirus.” Once integrated, the provirus can remain untranscribed until induced.

E. REPLICATION

When induced, DNA polymerase will attach to the provirus sequence and use the host cell’s machinery to transcribe the DNA, producing viral mRNA which binds to ribosomes to be translated into HIV proteins. First, small regulatory RNA is translated, followed by larger RNA that codes for viral enzyme and envelope/coat proteins. These proteins will serve as the building blocks that will assemble to make more HIV.

F. ASSEMBLY

Synthesized proteins and long HIV RNA move towards the membrane of the CD4 T cell. The virus is not infectious yet. At this point, HIV slightly buds out of the host cell.

G. RELEASE

Lastly, the immature virus buds out of the host cell. The lipid envelope like most cell membranes is a phospholipid bilayer. The HIV bilayer is formed by plasma membranes of previously infected cells in the earlier life cycle ventures of the virus. Carrying both viral and host glycoproteins helps the virus evade detection from our immune system. The most important cause behind viral infection is that it has evolved its own sugars and receptors in a way that mimics or interferes with the host glycan immune response (Banerjee and Mukhopadhyay, 2016). Here, the protease transcribed from the *Pro* gene splices the long viral RNA into sections,

each including vital genes or enzymes, that will serve as the genome of the new virus. Finally, the virus is now mature and infectious.

HIV has now hijacked our biosynthetic machinery to make more copies of itself. This cycle will continue indefinitely.

3. INFECTION AT THE CELLULAR LEVEL

A study done by Doitsh et. al (2010) showed the mechanism for how CD4 T cells are depleted in hosts remains poorly understood. Isolated human tonsil tissue was infected with HIV-1. Surprisingly, more than 95% of dying cells were not actively infected, they are considered “bystander cells.” The study showed that on day 6 of infection, the number of CD4 T cells were not altered. However, by day 9 the tissue was almost completely absent of CD4 T cells.

A. CASPASES AND IFI16

Infected HIV cells are brought to the lymph nodes, the “police stations” of our body along with non-infected CD4 T cells as an immune response. The role of lymph nodes and their pathology were recognized as important consequences of human immunodeficiency virus (HIV) infection since the beginning of the HIV epidemic (Dimopoulos, 2017). At this point, HIV attempts to infect the non-infected CD4 T cells in the lymph nodes. If unsuccessful, incomplete reverse transcripts accumulate in the cell.

The mass production is detected by the IFI16 sensor, which is on the lookout for rogue DNA that should not be in our cells. IFI16 is the only mammalian DNA sensor identified that recognizes both single and double-stranded (DS) DNA (Doitsh & Greene, 2016). IFI16 produces an inflammasome assembly, caspase 1 activation, and the induction of pyroptosis, a highly

inflammatory form of programmed cell death in these cells (Galloway et. al, 2015). On the other hand, infected cells activate caspase 3 and die from apoptosis (see figure 5).

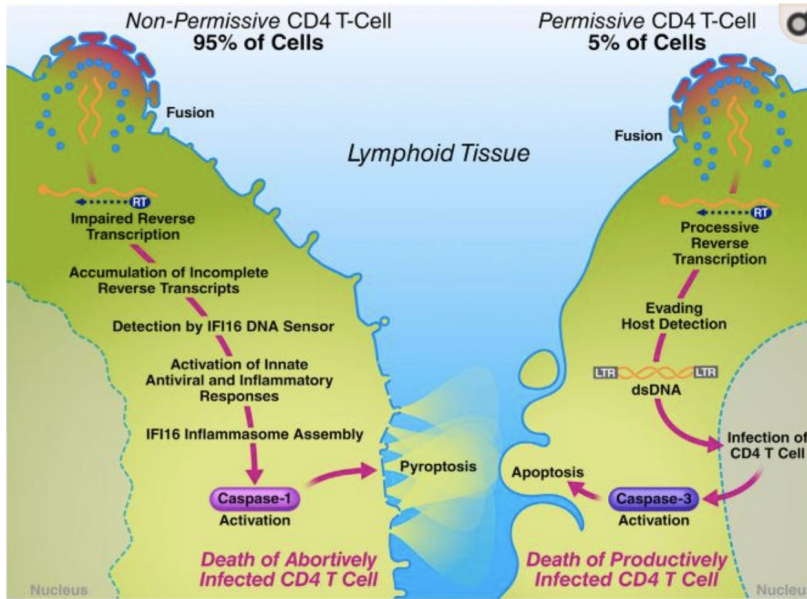


Figure 5- Caspase-3-Dependent Apoptosis and Caspase-1- Dependent Pyroptosis in CD4 T cell Death.

Source: Doitsh, G., & Greene, W. C. (2016). Dissecting How CD4 T Cells Are Lost During HIV Infection. *Cell host & microbe*, 19(3), 280–291. doi:10.1016/j.chom.2016.02.012

B. PROTEIN KINASES (PK)

When HIV uses integrase to incorporate its newly made DSDNA into our DNA, cellular sensor DNA-dependent protein kinases senses the breaks in DNA. DNA PK mounts response to bacterial and viral infection (Burma & Chen, 2004). DNA PK localizes very rapidly to DNA breaks and phosphorylates itself and other damage-responsive proteins, it appears that DNA-PK serves as both a sensor and a transducer of DNA-damage signals (2004). This too leads to programmed cell death.

C. CD8 T CELLS

CD8 T cells, like CD4 T cells, are generated in the thymus and express a T-cell receptor (TCR), a receptor that recognizes antigens on major histocompatibility complexes (MHC), and are a vital component in cellular immune response (Wissinger, n.d). When a cell is infected with

HIV, CD8 T cells are recruited to the lymph nodes, recognize the infected cells by a MHC dependent process, and are able to lyse cells harboring viral infection by the secretion of perforin and granzymes (Gulzar and Copeland, 2004). These cytotoxic T-lymphocytes (CTL), can also eliminate virally infected cells through the engagement of death-inducing ligands expressed by CD8⁺ T-cells with death receptors on the surface of the infected cell (2004). CD8 T cells also express perforin, a molecule that causes cell mediated toxicity after antigen specific stimulation.

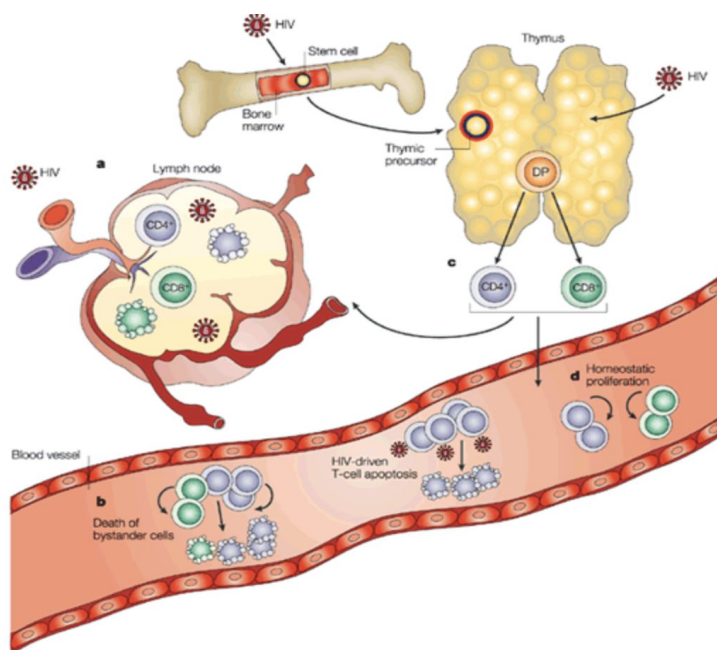


Figure 6- T cells during HIV infection.

Source: Gougeon, M. Apoptosis as an HIV strategy to escape immune attack. *Nat Rev Immunol* 3, 392–404 (2003) doi:10.1038/nri1087

A study done by Hersperger et al (2010) examined perforin expression in HIV infected individuals. They found that as HIV-specific performance-expression increased, viral load decreased. The capability for CD8 T cells to express perforin defines a novel correlation of control of HIV infection (2010). CTLs can also kill infected or malignant cells via cytokines tumor necrosis factor alpha (TNF- α) and interferon gamma (IFN- γ) or by T cell checkpoint Fas/Fas ligand that can activate a caspase response (Wissinger, n.d).

4. INFECTION AT THE ORGANISMAL LEVEL

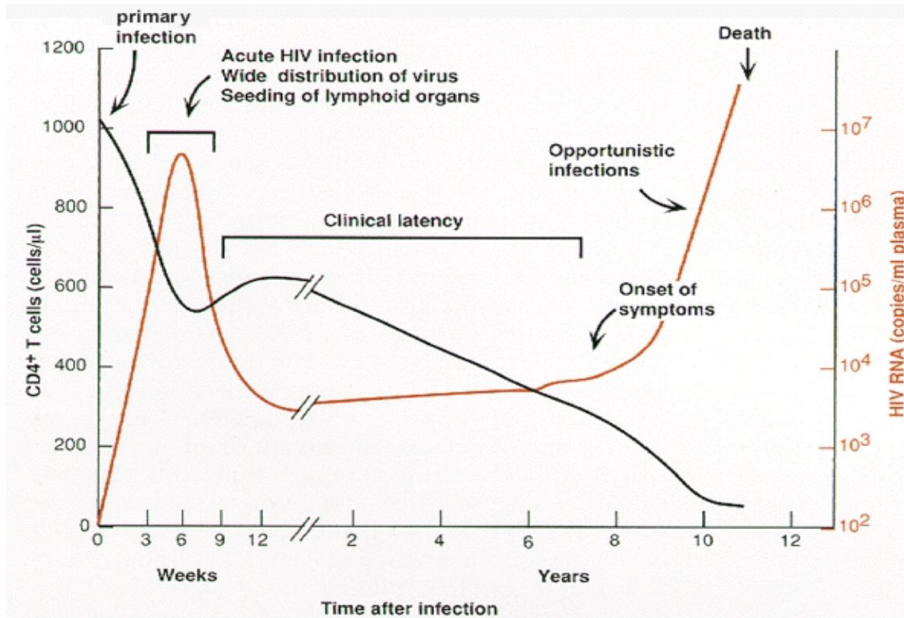


Figure 7- HIV Infection over years..

Source: Coffin JM, Hughes SH, Varmus HE, editors.

Retroviruses. Cold Spring

Harbor (NY): Cold Spring

Harbor Laboratory Press; 1997.

Course of Infection with HIV

and SIV. Available from:

[https://www.ncbi.nlm.nih.gov/b](https://www.ncbi.nlm.nih.gov/books/NBK19374/)

[ooks/NBK19374/](https://www.ncbi.nlm.nih.gov/books/NBK19374/)

A. PRIMARY INFECTION

Primary infection is characterized by a burst of viral replication that can be detected in the blood 3 weeks after infection (Clark et al. 1991; Daar et al. 1991; Tindall and Cooper, 1991). Some people experience flu-like symptoms during this time.

B. ACUTE INFECTION

Acute infection occurs about 3-6 weeks after infection and is characterized by increasing HIV RNA and significant decline of CD4 T lymphocytes in the peripheral blood 2-8 after infection (Gaines et al. 1990). CD4 T cells may rebound a small amount, but will rarely return to normal levels. This is also the point where a person infected has the highest likelihood of transmission. The probability of transmission is positively correlated with the viral burden in blood; every time the viral burden increases by a factor of 10, the risk of transmission increases

by a factor of 2.5 (Quinn et al. 2000). The risk of transmission is higher in patients with acute, early infection, than in patients with established infection (Wawer et al. 2005), partially because of the high viral load (see figure 7) and because of the homogeneity of the viral load that is clearly capable of causing infection (Cohen et al. 2011). At the organismal level, symptoms associated with acute HIV-1 infection are often too vague or nonspecific to lead to a diagnosis unless there is a high degree of suspicion (2011). Symptoms may include fever, malaise, pharyngitis, headache, diarrhea, maculopapular rashes, and meningoencephalitis (Coffin et al 1997).

C. CLINICAL LATENCY

After initial effects of HIV that are usually unnoticed, there is a long period referred to as “clinical latency” that lasts many years after primary infection (dependent on treatment). In this time, HIV is still active but reproduces at a low level, there is also a steady decline of CD4 T cells. However, people who take ART during this time can keep an undetectable viral load that has almost no risk of transmission. Symptoms in this period include fatigue, mild weight loss, generalized lymphadenopathy, thrush, and shingles (Coffin et al 1997).

A healthy person has about 1200 CD4+ T cells/ μ l. During latency, a patient's CD4 T cells continue declining. At about 500 CD4 T cells/ μ l, a patient may experience oral lesions, thrombocytopenia, basal cell carcinoma of the skin, and *Mycobacterium tuberculosis* (Coffin et al 1997).

D. AIDS

When CD4 T cells fall under 200 cells/ μ l, the patient has AIDS and is susceptible to AIDS-defining opportunistic infections and neoplasms (1997). These include: *Toxoplasma*

gondii, *Cryptosporidium*, *Treponema Pallidum*, *Candida albicans*, Kaposi's sarcoma, aseptic meningitis, myelopathies, myopathy, and AIDS dementia complex (Coffin et al 1997). HIV progresses until virtually all CD4 T cells are lost. Viral replication thrives as an outcome. A patient with AIDS does not die from AIDS itself, but by succumbing to these opportunistic infections and neoplasms (Coffin et al 1997).

5. DRUG THERAPY FOR HIV

After multiple decades and millions of deaths worldwide, an effective cure for HIV has not been found. Antiviral combination drug therapy (ART) was a major breakthrough because it effectively reduced the viral load and preserved immune function, prolonging the survival of HIV-infected patients. There are 7 main classes of drugs that can halt HIV at different stages in the HIV life cycle (see Figure 4). These include:

A. CCR5 ANTAGONIST

These drugs are designed to prevent HIV infection of CD4 T-cells by blocking the CCR5 receptor (Rao, 2009). Most patients have chemokine coreceptor CCR5, few have CXCR4. When the CCR5 receptor is unavailable, 'R5-tropic' HIV cannot bind with a CD4 T-cell to infect the cell (2009). Generic names of this drug include: maraviroc (MVC).

B. POST ATTACHMENT INHIBITORS

This design focuses on decreasing the flexibility of the CD4 receptor, hindering the access of CD4- bound gp120 to bind with CCR5 or CXCR4 due to its reduced stability (Henrich and Kuritzkes, 2013). Generic names of this drug include: ibalizumab-uiyk.

C. FUSION INHIBITORS

These drugs prevent membrane fusion by competitively binding to gp 41 and blocking the formation of the post fusion structure (Eggink et al, 2010). Generic names of this drug include: enfuvirtide.

D. INTEGRASE INHIBITORS

This design blocks the insertion of HIV DNA into the host cell's DNA. integrase is essential for retroviral replication, and the absence of integrase means that integrase inhibitors do not interfere with normal cellular processes, and therefore have a high therapeutic outcome (Pommier et al, 2005). Generic names of this drug include: Dolutegravir, and raltegravir.

E. NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIs)

These drugs bind directly to reverse transcriptase and inhibit reverse transcription (Sluis-Cremer et al, 2008). Generic names of this drug include: doravirine, efavirenz, etravirine, and nevirapine.

F. NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTIs)

This design uses DNA analogs to compete with the naturally occurring nucleosides that enable reverse transcription to prevent their transformation into viral RNA (Fowler et al, 2014). Generic names of this drug include: abacavir, emtricitabine, lamivudine, tenofovir disoproxil Fumarate, and zidovudine.

G. PROTEASE INHIBITORS

These drugs block the splicing of the long viral RNA accomplished by *pro* (Flexner, 1998). Generic names of this drug include: atazanavir, darunavir, fosamprenavir, ritonavir, saquinavir, and tipranavir.

H. DRUG THERAPY IN TODAY'S WORLD

These drugs have proven to be very successful, however, they can produce a lot of toxicity and viral resistance long-term (Von Laer et al, 2006). Long term complications of HIV-1 have recently been tied to the adverse effects of ART. Each drug class has been tied to its own side effects. To name a few, “nucleoside/nucleotide reverse transcriptase inhibitors are associated with lactic acidosis, lipodystrophy, and hyperlipidemia; non-nucleoside reverse transcriptase inhibitors are associated with neuropsychiatric symptoms, rash, liver toxicity, and lipid abnormalities; and protease inhibitors are associated with gastrointestinal intolerance and glucose and lipid abnormalities” (Ruest, 2011).

Aside from the side effects, however, ART has proven to keep CD4 T cell counts within a normal range and suppress HIV. Currently, there are 11 combination medications in pill form that only need to be taken once a day to suppress HIV and keep CD4 T cells within the normal range. Examples of these medications include Atripala, Striblid, Juluca, Symtuza and Dovato.

Even so, taking a pill everyday and being reminded of an HIV diagnosis can take its toll on just about anyone. In March of 2020, Canadian health officials announced approval of combination drug Cabenuva, a once a month injection for treatment of HIV-1 infection in adults whose viral load is suppressed (<50 HIV RNA copies/ mL or taking oral ART for at least 6 months) (Swindells et al, 2020). The drug contains cabotegravir (integrase inhibitor) and rilpivirine (non-nucleoside reverse transcriptase inhibitor).

In the study conducted by Swindells et al. (2020), patients were randomized to either continue oral ART or switch to monthly intramuscular injection of what has been trademarked “Cabenuva”. After 48 weeks of monitoring, results showed <50 HIV RNA copies/mL in 92.5%

of those taking Cabenuva, and in 95.5% taking oral ART (Swindells et al., 2020). There is little data on long-term toxicity and long-term side effects. Cabenuva has recently been denied FDA approval due to manufacturing issues. There is current research on Cabenuva being effective once every two months.

6. GENOME EDITING & INTRODUCTION TO CRISPR/CAS9

Genome editing was first introduced in 1962 by Szybalska and Szybalski, their study used the basis that cells require either dihydrofolate reductase (DHFR) or hypoxanthine phosphoribosyl transferase (HGPRT) to synthesize nucleic acids. Szybalska and Szybalski (1962) used a human bone marrow cell line D98S that were either HGPRT- or HGPRT+ (DHFR was inhibited in both), only HGPRT+ cells were able to synthesize DNA. HGPRT+ cell DNA was isolated and transformed into HGPRT- cells in a hypoxanthine-aminopterin-thymidine (HAT) medium, and the cells did not die. This was the first documented gene transfer in mammalian cell lines. Waclaw Szybalski is noted as the “Father of Gene Therapy.”

CRISPR/Cas9 is a gene editing technology that is revolutionizing biomedical research. Clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR associated protein 9 (Cas9) work hand-in-hand to correct errors in a genome, downregulate genes, and upregulate genes (Redman et al, 2016).

The mechanism behind CRISPR/Cas 9 happened naturally, as it was a way for bacteria to protect themselves from infection of viruses. When the bacteria detected viral DNA, it created a strand of DNA that matched that of the virus (CRISPR), and an endonuclease (Cas9) that cut the

viral genome when the guide DNA matched its target. This process disabled the virus, and therefore the bacterium avoided infection.

Researchers found that this breakthrough could be used in any cell type, including human cells. A CRISPR/Cas9 complex enters the nucleus and locks onto the Protospacer Adjacent Motif (PAM) which is a couple nucleotides downstream from the cut site and is required by the CRISPR/Cas9 complex (Importance of the PAM sequence in CRISPR experiments, n.d) (see figure 8). Once the target DNA is matched with the guide RNA (gRNA), Cas 9 makes the cut. Repair after the DNA cut may occur via two pathways: non-homologous end joining, typically leading to a random insertion/deletion of DNA, also known as a double strand break (DSB), inducing a mutation that will deactivate or “silence” the gene (Redman et al, 2016) (see figure 8).

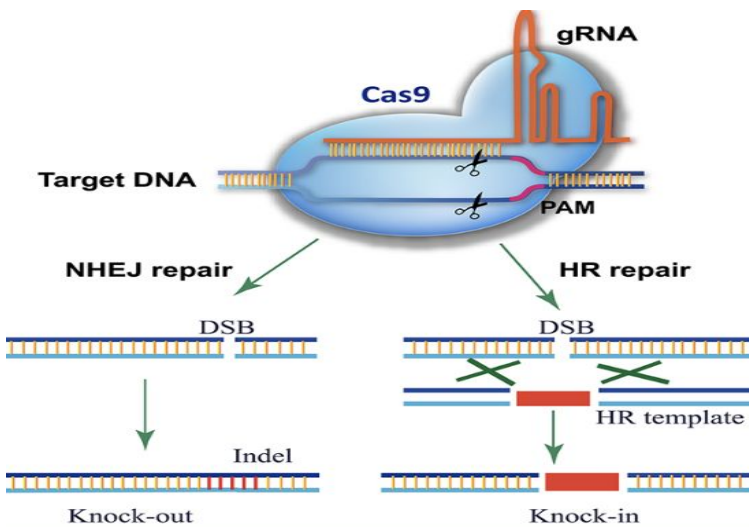


Figure 8- Mechanism of CRISPR/Cas9 genome editing.

Source: Ding Y, Li H, Chen L, Xie K, (2016, May 6). Recent Advances in Genome Editing Using CRISPR/Cas9. Retrieved from

<https://www.frontiersin.org/articles/10.3389/fpls.2016.00703/full>

Repair can also be done via homology directed repair (HDR) where a homologous piece of DNA is used as a repair template, this allows a healthy gene to replace the cut out mutant gene; thus, completing a precise genome modification (2016).

Current in-vitro research is focused largely on infectious diseases like HIV.

A. CRISPR/CAS 9 AND HIV

It is believed that latent infected immunal cells work as an HIV reservoir and allow for viral persistence, despite ART (Finzi et. al, 1997). A study done by Hu et al (2014) used HIV-1 gRNAs to stop long terminal repeat (LTR) transcriptional activity (important for HIV gene regulation) and eradicate proviral DNA from the latent myeloid cells in the brain that serve as an HIV reservoir.

The target gene was the HIV-1 LTR promoter U3 region. The U3 region contains the TATA box and several upstream transcription factor binding sites (TFBS) that control transcription (Opijen et al, 2004). Four gRNAs were synthesized (LTRs A-D). DNA oligonucleotides complementary to gRNAs A-D were placed into a “humanized Cas9 expression vector” (2014). Microglial cell line CHME5 was used, which contained a single, round, HIV-1 vector that also contained an enhanced green fluorescent protein (EGFP).

Histone deacetylases (HDAC) are natural occurring enzymes that remove the acetyl groups on a histone, allowing the histones to tightly wrap around the DNA, and therefore have a repressive influence on transcription (Haberland et al, 2009); the CHME5 cells were treated with trichostatin A (TSA), an HDAC inhibitor, reactivating transcription of the integrated provirus and expression of EGFP and HIV proteome (Hu et al, 2014).

Expressing the gRNAs plus Cas9 decreased the fraction of TSA-induced EGFP-positive CHME5 cells and caused a 190 bp deletion fragment between A and B target sites (Hu et al, 2014) (see figure 9).

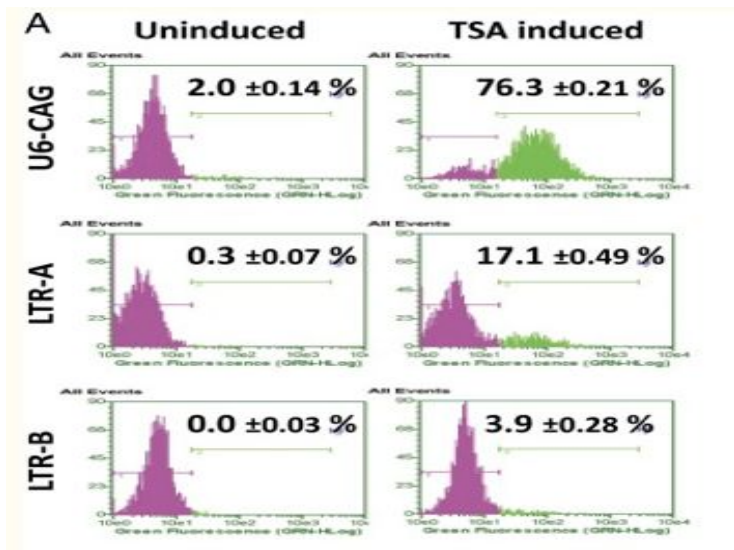


Figure 9- Reduction in TSA-induced EGFP-positive CHME5 cells after introduction of gRNAs and Cas9.

Source: Hu, W., Kaminski, R., Yang, F., Zhang, Y., Cosentino, L., Li, F., ... Khalili, K. (2014). RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. *Proceedings of the National Academy of Sciences of the United States of America*, 111(31), 11461–11466.

Furthermore, parent promonocytic cell line U-937 was infected with HIV-1 and derived subclone U1, which serves as an HIV-1 latency model for infected immune cells, exhibiting low-level constitutive viral gene expression that can be upregulated (Folks, et al 1987). U1 cells express the provirus in chromosome X (contains 9,709 proviral HIV bp and 226 flanking bp) and chromosome 2 (contains 9,709 proviral HIV bp and 467 flanking bp). In U1 cells that expressed LTR- A/B gRNAs and Cas9 there were 2 segments in chromosome x (only 833 bp and 670 bp) (see figure 10) and one segment in chromosome 2 (1,102 bp).

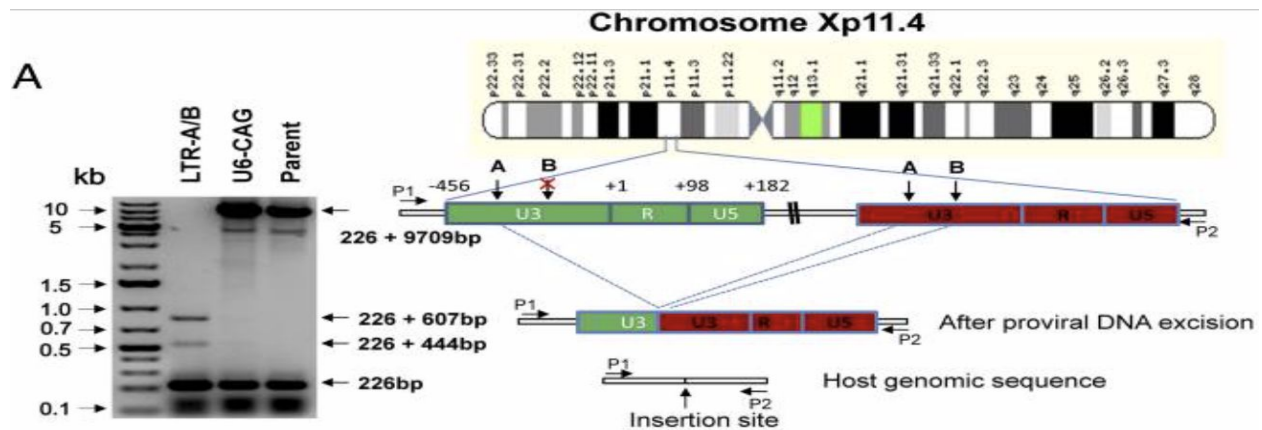


Figure 10- Cutting out HIV genome from U1 Xp11.4 chromosome.

Source: Hu, W., Kaminski, R., Yang, F., Zhang, Y., Cosentino, L., Li, F., ... Khalili, K. (2014). RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. *Proceedings of the National Academy of Sciences of the United States of America*, 111(31), 11461–11466.

The study concluded that gRNAs A/B enabled Cas9 to cut out the HIV 5'-3' LTR spanning viral genome segment in both chromosomes. The results of this study minimized toxicity while efficiently abolishing integrated HIV-1 provirus.

A recent experiment, done by Yu et al, 2018, simultaneously “knocked out” the CXCR4 and CCR5 genes in CD4+ T cells via CRISPR/Cas 9. As stated previously, chemokine coreceptors CCR5 and CXCR4 bind with either R5 tropic or X4 tropic HIV-1, respectively. There is a class of drugs that works as a CCR5 antagonist (see drug therapy section). However, these drugs create toxicity and therefore cause risk when a latent infection has affected the brain and other major neurological regions. There became a need to eradicate both co receptors on cells that are post infection. In their results, the efficiency of gene modification was 55% for CCR5 and 36% for CXCR4 CD4+ T cells (Yu et. al, 2018).

Furthermore, 9% of modified GHOST CXCR4+CCR5+ (cells with both co receptors) cells had disruptions in the CXCR4 and CCR5 loci (Hu et al, 2018). The results demonstrate the safety and efficiency of gene modification technology as a functional cure for infectious diseases like HIV-1.

B. siRNA AND HIV

Small interfering RNA (siRNA), a 21–23 nucleotide DSRNA responsible for post-transcriptional gene silencing, has attracted great interests as promising genomic drugs, due to its strong ability to silence target genes in a sequence-specific manner (Ku et al, 2016). In

1998, Fire et al. discovered that short DSRNA specifically regulates gene expression. A notable study done by Song et al (2005), designed a protamine-antibody fusion protein (F105-P) to deliver siRNA to HIV-1-infected or envelope transfected cells. In efforts to reduce viral replication via a dose dependent manner, they loaded the F105-P with *gag* siRNA. The results show that the proportion of productively infected cells declined from 85% to 36% when 1 nM of *gag* siRNA was added. Even with a ten-fold less siRNA, only 45% of cells were staining for HIV-1 *gag* (see figure 11).

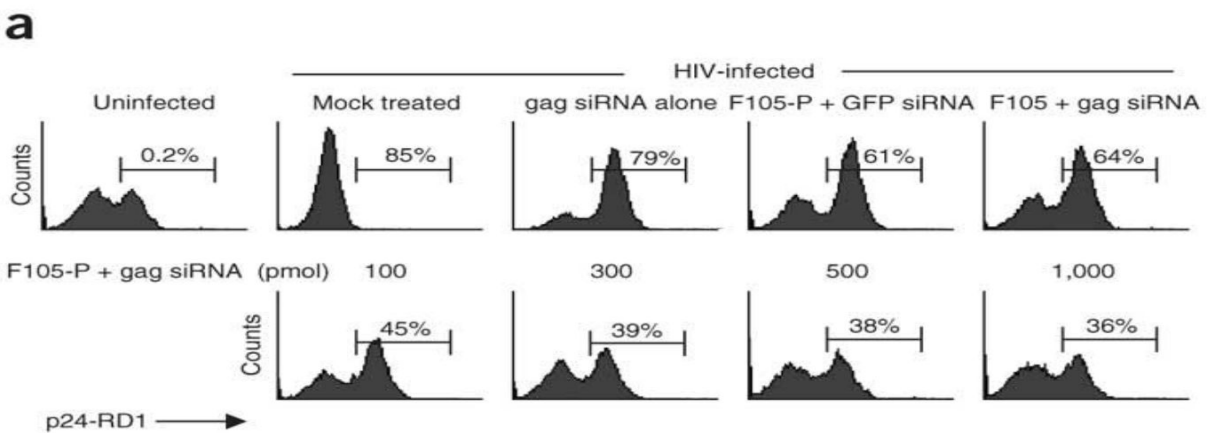


Figure 11- Treating HIV-1 infected cells with *gag* siRNA.

Source: Song, E., Zhu, P., Lee, S. *et al.* Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. *Nat Biotechnol* 23, 709–717 (2005). <https://doi.org/10.1038/nbt1101>

C. APTAMERS + siRNA AND HIV

Nucleic acid aptamers are oligonucleotides that function similar to antibodies by binding to a specific target molecule. Riboswitches are an example of naturally-occurring aptamers. RNA aptamers can be designed to target specific cell receptors while carrying and delivering siRNA. A study done by Zhou et al, 2009, synthesized covalent aptamer-siRNA chimeras (Ch A-1/ Ch B-68) to bind to gp120 and send siRNA to target *tat/rev*.

They also synthesized aptamers without siRNA (A1/B68) for the same task. Both aptamers and complexes were inserted in HIV-1 infected T lymphoblast cell derivative CEM T cells and primary blood mononuclear cells (PBMCs) and incubated over 9 days. A p24 antigen analysis was done on day 3,5,7 and 9 to determine if the aptamer and aptamer/chimera complex affected HIV-1 transcription. Results showed that all aptamer and aptamer/chimera complexes inhibited p24 production with the greatest inhibition done by aptamer/chimera complex A1 (see figure 12). Both studies demonstrate the effectiveness of siRNA inhibiting HIV propagation.

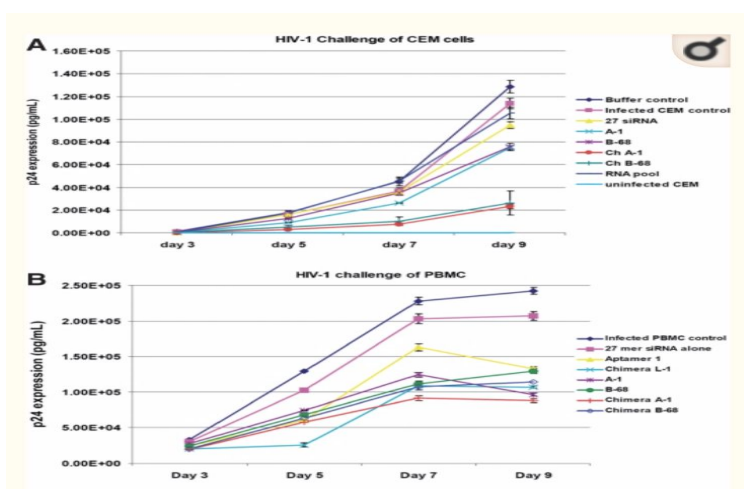


Figure 12- HIV-1 inhibition by gp120 aptamer and gp120-aptamer-chimera-complex.

Source: Zhou, J., Swiderski, P., Li, H., Zhang, J., Neff, C. P., Akkina, R., & Rossi, J. J. (2009). Selection, characterization and application of new RNA HIV gp 120 aptamers for facile delivery of Dicer substrate siRNAs into HIV infected cells. *Nucleic acids research*, 37(9), 3094–3109. doi:10.1093/nar/gkp185

D. siRNA AGAINST HIV REVERSE TRANSCRIPTASE

Reverse transcriptase (RT) is a primary target for antiviral therapies and drugs. In a study done by Surabhi and Gaynor (2002) two siRNA's were designed to target the HIV-1 RT gene (RT1 and RT2) and tested its efficacy in replication inhibition in MAGI cells (HeLa stem cell line derivative).

The MAGI cells were subjected to either RT1, RT2, tax siRNA (a control group for the study, human T-cell leukemia type 1 siRNA), and oligofectamine (a transfection reagent used to properly transfect eukaryotic cells with oligonucleotides and siRNA). After 6 days, the cultures

were assayed for the p24 antigen levels. Transfection of either RT1 or RT2 siRNA inhibited HIV-1 replication in MAGI cells by >90% compared to tax siRNA or oligofectamine alone (Surabhi and Gaynor, 2002).

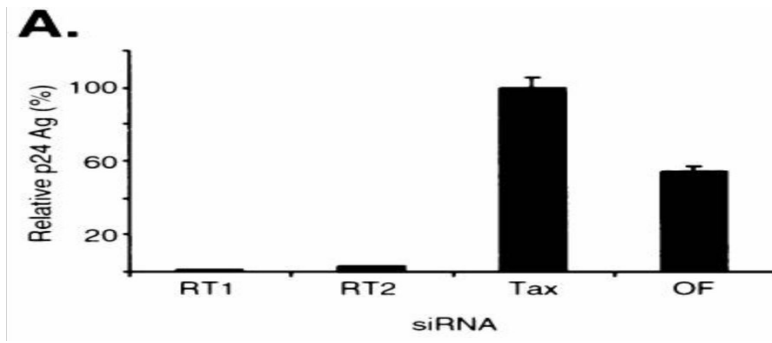


Figure 13- Assay of p24 antigen in MAGI cells transfected with RT 1, RT2, Tax (control), and OF (control)

Source: Surabhi, R. M., & Gaynor, R. B. (2002). RNA interference directed against viral and cellular targets inhibits human immunodeficiency Virus Type 1 replication. *Journal of virology*, 76(24), 12963–12973. <https://doi.org/10.1128/jvi.76.24.12963-12973.2002>

E. GENOME EDITING IN TODAY'S WORLD

CRISPR/CAS 9 differs from RNAi (siRNA/ shRNA) because CRISPR/CAS 9 has the ability to generate substitute genes (knock-in), fix a gene, and integrate a gene at a specific locus— not discussed since HIV-1 requires a “knock-out.” There are far less concerns about RNAi than about CRISPR/ CAS 9.

CRISPR/ CAS 9 has proven to be a revolutionary tool in biomedical research and has extreme potential in gene editing therapies. Previous explorations of CRISPR/CAS 9 were mainly conducted *in vitro* or in animal germlines, the translatability is limited to: tissues with adult stem cells susceptible to culture and manipulation or currently impermissible due to ethical concerns (Dai et al., 2016). Recently, three studies (Tabebordbar, et al., 2016; Nelson, et al., 2016; Long, et al., 2016) delivered CRISPR/ CAS 9 through intramuscular, intraperitoneal or intravenous injection to correct a nonsense mutation in the dystrophin gene that leads to Duchenne Muscular Dystrophy (DMD) in mouse models, resulting in restoration of dystrophin

expression and muscle function. CRISPR/ CAS 9 HDR was also delivered through intravenous injection to mice with human liver disease (Yang et al., 2016; Yin et al., 2016).

The viral vectors used are adeno-associated viruses (AAVs) which is a non-pathogenic SSDNA virus that can insert site-specific genetic material on chromosome 19 with very high certainty. Chromosome 19 has the highest gene density of all human chromosomes (Grimwood et al, 2004). In 2019, the FDA approved clinical trials on patient volunteers in three areas; cancers, blood disorders, and eye disease; all of which mean no genetic change can be passed onto offspring.

There are many challenges to CRISPR/ CAS 9 *in-vivo*. Specificity of CRISPR/CAS 9 can cause off-target side effects, which may lead to uncontrollable consequences such as malignant transformation (Dai et al., 2016). This challenge can be reduced by modifying Cas9 to a high fidelity (Kleinstiver et al, 2016), or optimizing the sgRNA (Doench et al., 2016). Other challenges include (1) AAVs themselves have a small genome and therefore have a limited cargo capacity (2) HDR-mediated repair can require patient-specific design of sgRNAs and donor templates, which can challenge the mass-production and cost of this therapy (Dai et al., 2016).

Some challenges to CRISPR/Cas9 are also challenges to RNAi such as (3) translatability to in-vivo delivery (4) immunogenicity and (5) fitness of edited cells.

In 2018, FDA approved Onpattro (patisiran), an RNAi drug used for the treatment of peripheral nerve disease (polyneuropathy) caused by hereditary transthyretin-mediated amyloidosis (HATTR) which causes buildup of abnormal amyloid protein in peripheral nerves and is often fatal (FDA, 2018). Onpattro has siRNA that targets the abnormal protein

transthyretin (TTR) which reduces the accumulation of amyloid deposits (2018). An annual course of this drug costs \$450,000, making affordability and access a major issue.

Perhaps the biggest challenge to CRISPR/CAS 9 genome editing is not physical, but ethical. In 2018, Chinese doctor Jian Kui He claimed to have created the first gene-edited babies, designed to be naturally immune to HIV-1 by disabling the CCR5 gene via CRISPR/CAS 9 (Li et al., 2019). This created an uproar worldwide, especially because other effective, cheap and more accessible treatments are available to prevent transmission of HIV-1. China explicitly banned clinical procedures of gene-editing on human embryos for reproductive purposes (2019). Hu was sentenced to three years in prison for illegal practices.

Many people struggle with the concept of gene editing and believe that genome editing for therapeutic purposes will then begin use for non-therapeutic uses and “enhancements.” Furthermore, gene editing on germ-line cells has been a topic of much debate, since in-vitro fertilization (IVF) and preimplantation genetic diagnosis (PGD) is available (What are the ethical concerns of genome editing, 2017). Although there is an all-too-real threat of “designer babies,” IVF and PGD simply may not be enough for parents who are homozygous for a disease-causing variant.

Other concerns unique to CRISPR/CAS 9 include (1) future generations that have been affected by the germline editing did not provide consent (2) future effects on the germline are unknown (3) the technology will be available only to the wealthy, creating a society defined by the quality of their altered genome (2017).

7. CYTOTHERAPY INTRODUCTION

Cytotherapy, or cell therapy, is the transplantation of cells to replace damaged tissue and/or cells (Facts about cellular therapies, n.d). The first successful blood transfusion occurred in 1818 by James Blundell, MD to a patient that was hemorrhaging during childbirth. In 1931, Paul Niehans, MD injected bull parathyroid gland cells into a patient suffering from seizures. Both occurrences yielded successful results. Furthermore, research suggests the field has tremendous potential to treat a plethora of diseases.

A. STEM CELLS AND HIV

Stem cells are precursors to all cell types. They are undifferentiated and can form many cell types including some nerve and cardiac cells. Hematopoietic stem cell (HSCs) transplantation, or bone marrow transplantation (BMT), is the most common cell therapy and is used to treat many blood conditions like leukemia and sickle cell anemia (Facts about cellular therapies, n.d). HSCs are capable of forming red blood cells, platelets, and white blood cells (immune system cells) (see figure 14).

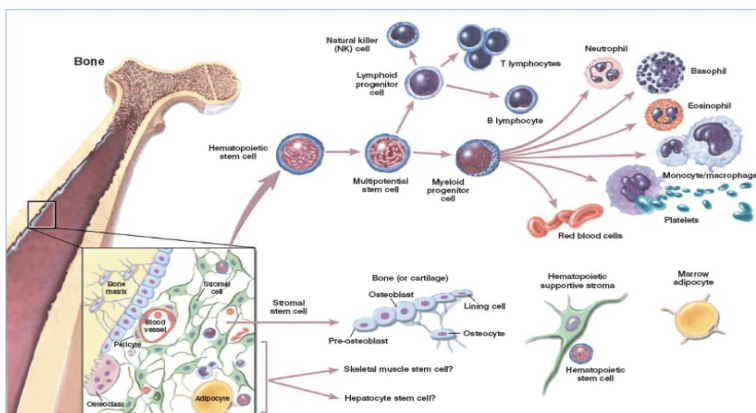


Figure 14- Hematopoietic stem cell differentiation.

Source: © 2001 Terese

Winslow (assisted by Lydia

Kibiuk)

In 1956, the first successful bone marrow transplant was performed by E. D. Thomas, MD. The donated HSCs replaced the hematopoietic system of the recipient over time. It's also

important to note that human leukocyte antigen (HLA) matching is necessary for blood and bone marrow transplants. HLAs are membrane surface markers that the immune system uses to recognize foreign cells. The higher the HLA match between donor and recipient, the likelier the transplant will be successful.

A single HSC is capable of reestablishing the entire blood forming system of an irradiated mouse (Karp et al, 2018). Furthermore, saving the umbilical cord blood of a newborn baby provides a source of HSCs if they are needed later (2018). Stem cells can also be collected from peripheral blood (PB) using a harvesting machine.

The first person has been cured of HIV-1 via bone marrow transplant. Homozygosity for a 32 base pair deletion in the CCR5 (CCR5 delta 32) allele results in an inactive CCR5 gene, defects in HIV-1 coreceptor leads to resistance of HIV-1 infection (Liu et al., 1996). In a study by Hütter et al., (2009) stem cells were transplanted from a donor who was homozygous for CCR5 delta 32 (CCR5 delta32/delta32) deletion into a patient with both acute myeloid leukemia (AML) and HIV-1 infection.

After stem cell transplantation (SCT) there was no viral rebound and the patient discontinued ART (see figure 15). CCR5 delta 32 deletion is observed in approximately 1% of the white population, and offers a natural resistance to HIV-1 infection (Hütter et al., 2009). It is important to note this method would not be successful with CXCR4 tropism. Essentially, blood cells were replenished with healthy cells, the immune system was “replaced” as his resulting T cells were immune to HIV invasion.

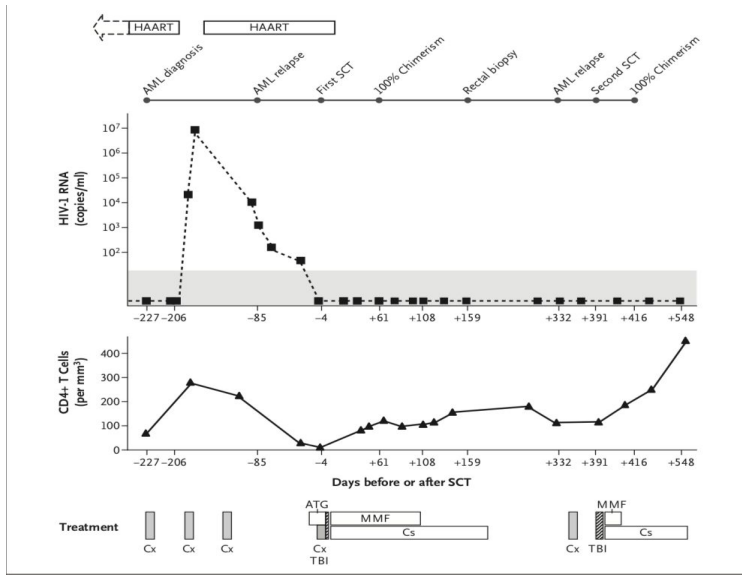


Figure 15- Treatment course of AML and HIV-1 via PCR after SCT.

Source: Hütter, G., Nowak, D., Mossner, M., Ganepola, S., Müßig, A., Allers, K., ... & Blau, I. W. (2009). Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *New England Journal of Medicine*, 360(7), 692-698.

B. CAR STEM CELLS AND HIV

The primary purpose of a chimeric antigen receptor (CAR) is to direct a T cell to specifically target an antigen of interest (Zhen et al., 2017). A study done by Kitchen et al, (2009) genetically programmed a chimeric antigen receptor (CAR) in HSCs that successfully turned into mature CD8+ cytotoxic T lymphocytes that expressed an anti-HIV T cell receptor (TCR). The transduction directed the maturation of a large quantity of polyfunctional, HIV specific CD8+ cells capable of recognizing and killing HIV cells in mice (2009). The result was a significant % of HIV cell lysis (see figure 16).

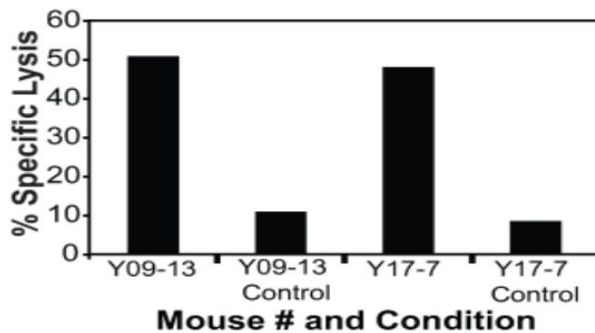


Figure 16- Lytic activity of modified HSCs. Source: Kitchen, S. G., Bennett, M., Galić, Z., Kim, J., Xu, Q., Young, A., Lieberman, A., Joseph, A., Goldstein, H., Ng, H., Yang, O., & Zack, J. A. (2009). Engineering antigen-specific T cells from genetically modified human hematopoietic stem cells in immunodeficient mice. *PLoS one*, 4(12), e8208. <https://doi.org/10.1371/journal.pone.0008208>

C. HUMAN EMBRYONIC STEM CELLS (hESCs)

Human embryonic stem cells differ in that they are nonautologous and require an embryo donor. HESCs are isolated from blastocysts and are pluripotent, which give rise to all somatic cell types in the embryo (Vazin and Freed, 2010). The farther a cell is in development, the less pluripotent it is. HESCs have been isolated from embryos via IVF.

A small tissue is taken from the patient, one somatic cell is fused with donor enucleated oocyte. The resulting oocyte that has the patient's nucleus develops into an early embryo, and ES cells are harvested and grown in culture. ES cells are induced to differentiate into the required cells, which get transplanted into the patient (see figure 17).

HESCs are induced with specific mediums depending on the needed cell type. Generation of transplantable motor neurons occurs with addition of retinoic acid in the medium (Li et al., 2005).

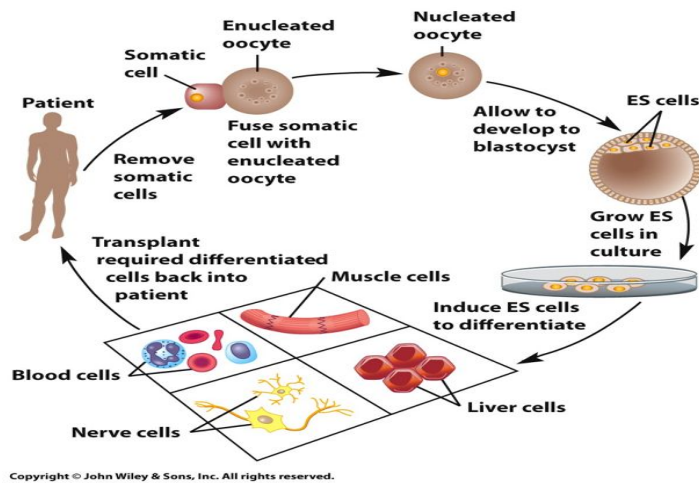


Figure 17- Mechanism to obtain differentiated hESC.

Source: Karp, G., Iwasa, J., & Marshall, W. (2018, May 10). Karp's Cell Biology, 8th Edition, Global Edition. Retrieved from [https://www.wiley.com/en-cw/Karp's Cell Biology, 8th Edition, Global Edition-p-9781119456292](https://www.wiley.com/en-cw/Karp's+Cell+Biology,+8th+Edition,+Global+Edition-p-9781119456292)

After the study done by Hütter et al., (2009), a search for an unlimited source of CCR5 delta 32 deletion cells began. In a study done by Pomerantseva et. al, (2011), 137 hESC lines

were tested (from the Reproductive Genetics Institute's hESC lines collection), and found 12 hESC lines with CCR5 delta 32 deletions, 11 heterozygous and 1 homozygous (see figure 18).

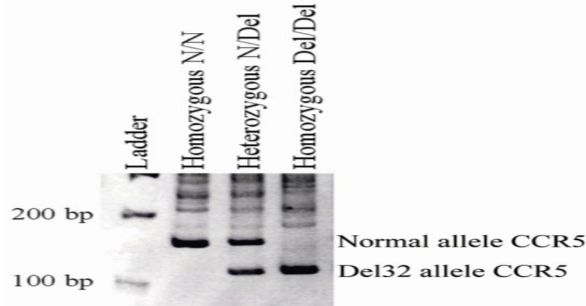


Figure 18- gel electrophoresis testing for CCR5 delta 32 deletion

Source: Pomerantseva, E., Kukhareenko, V., Goodman, A., Vrlinsky, O., Rechitsky, S., & Kuliev, A. (2011). Human embryonic stem cell lines with CCR5-del32 allele conferring resistance to HIV. *Stem Cell Discov*, 1, 67-70.

The possibility of unlimited quantities of these cells will overcome many obstacles. First and foremost the need for a bone marrow transplant will be eradicated, which is a painful, long procedure that may cause later complications. Also, a study by Taylor et al. (2005) emphasized that only 10 highly selective donors could provide the maximum practical benefit for HLA matching. Therefore, HIV-1+ patients that do not have blood cancers may be eligible for said treatment.

D. INDUCED PLURIPOTENT STEM CELLS (iPSCs)

Induced pluripotent stem cells (iPSCs) are similar to hESC but do not require the use of an embryo. The discovery of iPSCs revolutionized what was thought about cell differentiation. It was believed that cell differentiation was irreversible, once a liver cell became a liver cell, it could never turn into any other cell. A study done by Yamanaka and Takahashi (2006) reprogrammed a mouse connective tissue fibroblast into a pluripotent stem cell by introducing 4 genes novel to embryonic stem cells (Oct3/4, Sox2, c-Myc, and Klf4). The study proved pluripotent stem cells could be generated from a fully differentiated cell, and completely revolutionized biomedical research. IPSc have been used to correct sickle cell anemia in mice.

A study done by Higaki et al. (2018) used iPSCs transduced with short-hand pin RNA (shRNA) lentivector to target the HIV-1 promoter region (shPromA) to halt HIV-1 replication. The treated iPSCs successfully differentiated into macrophages. As mentioned in the gene therapy section, RNAi uses a sequence specific guide. In order to confirm that the transcriptional suppression was sequence specific and not off-target silencing, the scientists created shPromA and shPromA-M2, that has a two nucleotide mismatch from shPromA (Higaki et al., 2018). The iPSC-derived macrophages were subjected to CCR5-tropic HIV-1 infection (2018).

Transcriptional activity was measured at day 4 and day 11, iPSC-derived macrophages transfected with shPromA inhibit HIV-1 infection, 10 time reduction at day 4 and 20 time reduction by day 11 (see figure 19). Similarly, reverse transcriptase activity was measured and similar results were established.

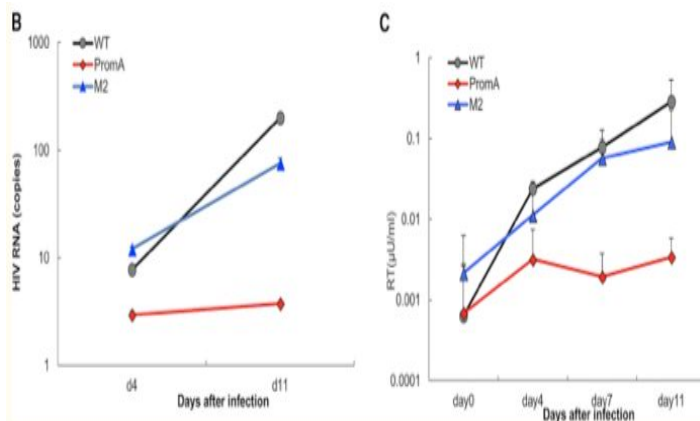


Figure 19- reduction in HIV replication in iPSC- derived macrophages expressing shPromA, shPromA-M2, and control (WT).

Source: Higaki, K., Hirao, M., Kawana-Tachikawa, A., Iriguchi, S., Kumagai, A., Ueda, N., Bo, W., Kamibayashi, S., Watanabe, A., Nakauchi, H., Suzuki, K., & Kaneko, S. (2018). Generation of HIV-Resistant Macrophages from iPSCs by Using Transcriptional Gene Silencing and Promoter-Targeted RNA. *Molecular therapy. Nucleic acids*, 12, 793–804. <https://doi.org/10.1016/j.omtn.2018.07.017>

In a study done by Kambal et al (2010), human hematopoietic stem cells (HSCs) were generated into anti HIV-1 iPSCs (containing lenti viral CCR5 shRNA and human/rhesus chimeric TRIMα gene) that differentiated into HIV-1 resistant immune cells. The TRIMα gene was generated by replacing stretch of 11 human amino acids with 13 amino acids from the rhesus

macaque essential for HIV-1 restriction (Kambal et al., 2010). The derived anti-HIV macrophages, wild type (control) peptide (WT), and enhanced green fluorescent protein (EGFP) were cultured with CCR5 tropic HIV-1 and assayed for p24 antigen postinfection.

Results show that at post-infection day 15, there was a over a 2-log reduction in p24 antigen in the anti-HIV macrophages than the WT and EGFP cultures (Kambal et al., 2010) (see figure 20). By generating a sufficient quantity of iPSCs expressing the anti-HIV genes, the cells can replace the immune system with HIV- resistant immune cells.

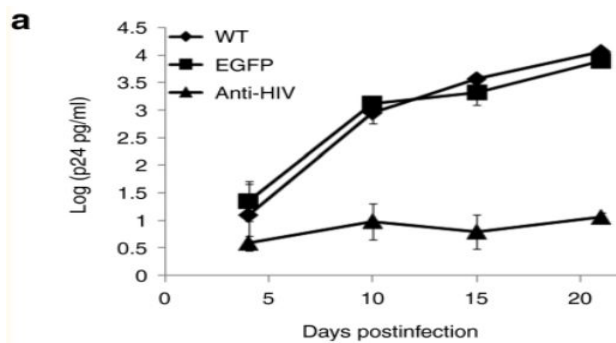


Figure 20- reduction in HIV replication in iPSC- derived macrophages expressing anti-HIV genes.

Source:Kambal, A., Mitchell, G., Cary, W., Gruenloh, W., Jung, Y., Kalomoiris, S., Nacey, C., McGee, J., Lindsey, M., Fury, B., Bauer, G., Nolte, J. A., & Anderson, J. S. (2011). Generation of HIV-1 resistant and functional macrophages from hematopoietic stem cell-derived induced pluripotent stem cells. *Molecular therapy : the journal of the American Society of Gene Therapy*, 19(3), 584–593.
<https://doi.org/10.1038/mt.2010.269>

E. iPSC-NKs, hESC-NKs, PB-NKs, UCB-NKs AND HIV

Natural killer cells are vital in controlling HIV-1 propagation. NK cells are part of the innate immune system, meaning they do not require previous exposure to an antigen, and instead recognize antigens on MHCs. NK cells, B lymphocytes, and T lymphocytes (all WBC) are differentiated from lymphoblasts. In a study done by Ni et al. (2011), hESCs, peripheral blood (PB) cells, umbilical cord blood cells (UCB), and iPSCs were differentiated into NK cells and successfully suppressed HIV replication. The stem cells successfully differentiated into NK cells, as tested by cell surface markers and cytolytic activity against tumor cells. CEM-GFP T cell line was infected with HIV-1 NL4-3 and then cultured with each stem cell population (Ni et al., 2011). Quantification of p24 gag protein in the untreated cultures and treated co cultures show

the reduction in HIV-1 activity via stem cell differentiation (see figure 21) at different effector-to-target cell (E:T) ratios.

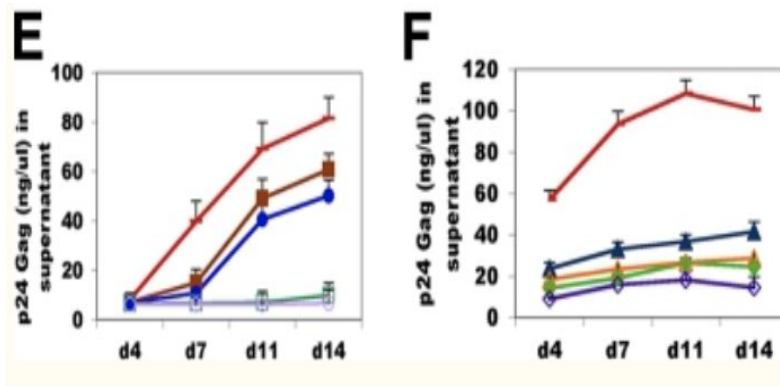


Figure 21- reduction in HIV replication in stem cell co cultures. (E) Red: no treatment; 1:1 ET- UCB-NK cells: maroon, hESC-NK cells: blue; 10:1 ET- UCB-NK cells: dark green, hESC-NK cells: lilac. (F) Red: no treatment; 1:1 ET- iPSC-NK cells: dark blue, PB-NK cells: light green; 5:1 ET- iPSC-NK cells: orange, PB-NK cells: purple. Source: Ni, Z., Knorr, D. A., Clouser, C. L., Hexum, M. K., Southern, P., Mansky, L. M., Park, I. H., & Kaufman, D. S. (2011). Human pluripotent stem cells produce natural killer cells that mediate anti-HIV-1 activity by utilizing diverse cellular mechanisms. *Journal of virology*, 85(1), 43–50. <https://doi.org/10.1128/JVI.01774-10>

In the same study, co-NK cells (hESC-NK, UBC-NK, iPSC-NK, PB-NK) were incubated with HIV-1 infected CD4+ T cells, and levels of CD107a and CD56 expression were measured (proteins that are expressed during NK cytolytic activity) (Ni et al., 2011). At day 7 to 10 of HIV-1 infection, CD levels were assayed. The results show a significantly higher percentage of CD activity in cultures with co-NK cells and HIV-1 infected CD4+ T cells versus co-NK cells with noninfected CD4+ T cells (see figure 22). All co-NK cells provided cytolytic activity against HIV-1 infected CD4+ T cells.

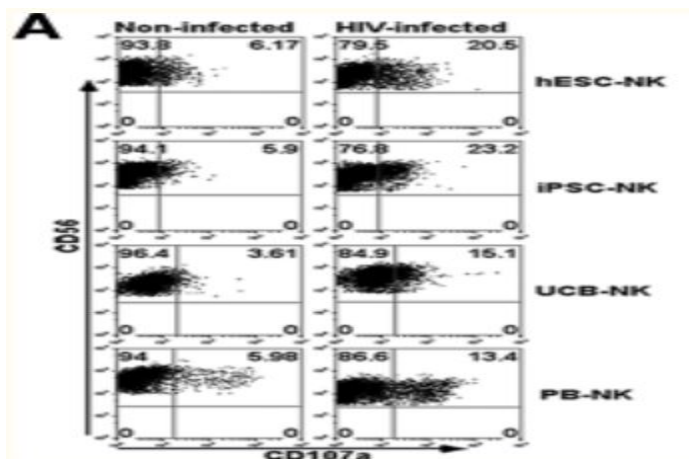


Figure 22- co-NK cell cytolytic activity against HIV-1 infected CD4+ T cells. Source: Ni, Z., Knorr, D. A., Clouser, C. L., Hexum, M. K., Southern, P., Mansky, L. M., Park, I. H., & Kaufman, D. S. (2011). Human pluripotent stem cells produce natural killer cells that mediate anti-HIV-1 activity by utilizing diverse cellular mechanisms. *Journal of virology*, 85(1), 43–50. <https://doi.org/10.1128/JVI.01774-10>

F. CYTOTHERAPY IN TODAY'S WORLD

Today, stem cells that come from bone marrow or in blood transplant are used in transplant procedures to treat patients with cancers and disorders of the blood and immune system (FDA Warns About Stem Cell Therapies, n.d). The only stem cell based products that are FDA-approved in the US are hematopoietic progenitor cells derived from cord blood (FDA warns about Stem Cell Therapies, n.d). Examples of diseases treatable with FDA approved stem cell procedures include, but are not limited to: leukemias, lymphomas inherited RBC or platelet abnormalities, neutropenias, phagocyte disorders, lysosomal storage disorders, and myeloproliferative disorders (Stem cell Treatments and Products Approved by the FDA, 2019). A bone marrow transplant costs an average of \$193,000 per patient (Westerman and Bennett, 1996). Despite FDA regulations, clinics have begun using autologous stem cell therapies to treat knee pain and arthritis.

There are many concerns regarding stem cells, including the failure of cells to work as expected. In other words, the cell may have acquired a few characteristics of the new cell type but not any new function (Catacchio, 2013). Mesenchymal stem cells from adipose tissue (MSC) derived neurons exhibited synaptic transmission, but no evidence proved that activity was modulated by neurotransmitters (Cho et al., 2005). Hence, it is critical to prove required cellular activity by means of experimentation in *in-vivo*-like environments.

Perhaps the largest concerns of stem cell therapy are hematological (including AML and chronic myeloid leukemia) and non-hematological (teratoma and non-teratoma tumors) (Anisimov et al., 2010).

Hematological malignancies can occur due to donor stem cells undergoing blast transformation under certain factors in the recipient, thus causing *de novo* malignancies (2010). A study done by Thomas et al., 1972 witnessed leukaemic transformation of engrafted human marrow cells *in-vivo*. Another mechanism that can lead to post-transplantation hematological malignancies is donor stem cells may have already transformed before the moment of transplantation (Anisimov et al., 2010). It was demonstrated by Glasser et al., (2010) both the recipient and donor synchronously developed AML after bone marrow transplant. This can be due in part to a genetic predisposition of stem cells to blast transformation (Anisimov et al., 2010).

Non-hematological malignancies can also occur after stem cell transplantation. The likely mechanism is PB stem cells and bone marrow stem cell material can be contaminated with metastatic donor cells able to cause tumors in recipients. There have been several cases of Kaposi sarcoma development in stem cell recipients (Avital et al., 2007; Helg et al., 1994; Palencia et al., 2003).

In order to prevent malignancies, prolonged differentiation (culturing cells for a prolonged period of time versus short period of time pre-implantation) may be key (Anisimov et al., 2010). A matter of a few more days *in-vitro* can decrease the proliferation potential and pluripotency of the cells and, therefore, the risk of teratoma/ tumor growth (Anisimov et al., 2010).

Another mechanism that may be vital in preventing malignancies is the introduction of suicide genes, that will activate apoptosis if the grafted cells become tumorigenic (Anisimov et al., 2010). A study done done by Schuldiner et al. (2003), transduced herpes-simplex-virus

thymidine kinase (HSV-TK) in hESCs prior to implantation, and tumor growth was stopped and eliminated after treatment of FDA approved ganciclovir (induces destruction of HSV-TK+ cells and is non lethal to other cell types).

There is a large ethical concern for the use of hESCs. A doctor at the Harvard Stem Cell Institute emphasized that opponents argue the research is unethical, because deriving the stem cell destroys the blastocyst, an unimplanted human embryo at day 6-8 of development (Examining the ethics of embryonic stem cell research, n.d). He also stated however, it is important to note that the embryo is not implanted and growing in a woman's uterus, it is a blastocyst, a cluster of 180-200 cells growing in a petri dish (Examining the ethics of embryonic stem cell research, n.d).

Lastly, there is also concern of a residual memory in iPSCs. In a study done by Kim et al. (2010), iPSCs harbored residual DNA methylation signatures characteristic of their somatic tissue of origin. This "epigenetic memory" is favorable if the differentiation is along lineages related to the donor cell, but unfavorable if the differential is a completely different cell type (Kim et al., 2010). This memory can be reset however, with chromatin modifying drugs.

8. INTRODUCTION ON MEDICINAL MARIJUANA

Cannabis was first cultured approximately in the third millennium B.C in central and south Asia (Marijuana History, 2019). In its early days, cannabis was used to: treat sore eyes (China), anesthetize (India), and treat earaches (Greece). Cannabis was brought to the US in the early 1600's when Jamestown settlers brought the marijuana plant from Europe (Marijuana History, 2019).

There are approximately 100 cannabinoids in the cannabis plant, including tetrahydrocannabinol (THC), which is thought to be the main psychoactive component (Abrams and Guzman, 2015). It was found that cannabinoids have a receptor-mediated mechanism; in 1988 the first cannabinoid receptor (CB1) was identified in the brain by attaching a radio-label to synthetic cannabis (Abrams and Guzman, 2015). The protein is present in essentially all tissues.

What binded to this protein channel remained a mystery until 1992, when the first endocannabinoid, anandamide, was discovered. Thus confirming the body's endocannabinoid system. Endocannabinoids function as neuromodulators, the ligands are in the presynaptic nerve terminals, binding the endocannabinoid to CB1 activates the G protein, which opens potassium channels (decrease cell firing), and opens calcium channels (decrease neurotransmitter release) (Abrams and Guzman, 2015) (see figure 23).

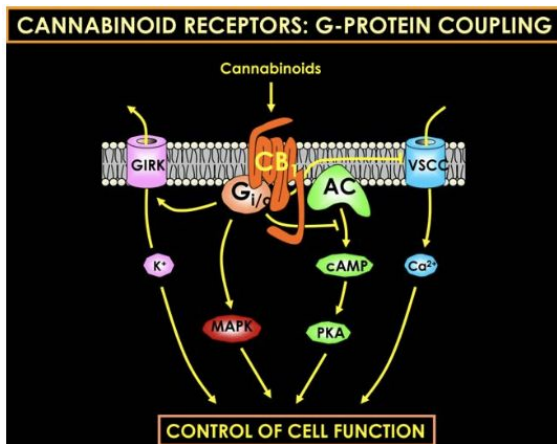


Figure 23- G protein coupling with Cannabinoid receptor

Source: Abrams, D. I., & Guzman, M. (2015). Cannabis in cancer care. *Clinical Pharmacology & Therapeutics*, 97(6), 575-586.

The biological functions “relax, eat, sleep, protect and forget” summarizes the various functions of the endocannabinoid system (Temple, 2016). In 1993, CB2 was discovered outside of the brain, with the highest abundance of receptors on the B lymphocytes and NK cells, suggesting a role in immunity (Abrams and Guzman, 2015).

A. MEDICINAL MARIJUANA AND HIV

HIV-1 attacks the immune system and begins a life-long battle with the host's body. A person with HIV (and ultimately AIDS) can experience AIDS wasting syndrome, nerve damage (pain to the touch), fatigue and nausea (usually related to medication), depression and anxiety. Marijuana, some patients say, erases all of these problems and then some (Joy and Mack, 2000).

ART drugs can make the lives of people with HIV miserable. ART drugs (especially protease inhibitors) are known to make people very sick, nausea, diarrhea, loss of appetite, vomiting, and more become a way of life for some- very similar to that experienced by cancer patients during chemotherapy (Joy and Mack, 2000).

The CDC defines AIDS wasting syndrome as the involuntary weight loss of more than 10% of one's weight, accompanied by diarrhea or fever that lasts more than 30 days. For people with HIV-1, loss of 5% of their body weight appears life-threatening (Joy and Mack, 2000).

Wasting occurs by two processes, cachexia and starvation. Cachexia results from disproportionate loss of lean tissue mass; Starvation results from food and/or nutrient deprivation. Starvation in people with HIV-1 is usually seen from a loss of appetite due to ART drugs, opportunistic infections that result in ulcers in the mouth, throat and esophagus that make eating difficult, opportunistic infections that result in extreme diarrhea, and/or the overgrowth of microbes that naturally live in the digestive tract (Joy and Mack, 2000).

A double-blind study done by Beal et al. (1995), randomized 139 patients experiencencing AIDS related anorexia to either receive 2.5mg dronabinol (synthetic THC cannabinoid) twice a day or the placebo. Dronabinol was associated with increased appetite in just 2-4 weeks of treatment, and the differences between drug and placebo were significant after

4 weeks of treatment (Beal et al., 1995) (see figure 24). Improvement in mood and decreased nausea was also significant in patients who received dronabinol. Since increased appetite was noted about 2-4 weeks after treatment (6 week study), there was limited time to analyze weight gain/loss of either part, however, weight results still diverged between dronabinol and placebo treated patients (Beal et al., 1995) (see figure 25). After this study, the FDA approved dronabinol, under the trademark Marinol, as a treatment for anorexia in patients with AIDS.

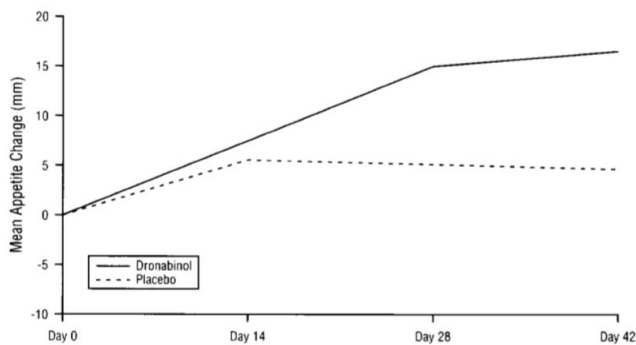


Figure 24- Mean appetite change in dronabinol and placebo patients.

Source: Beal, J. E., Olson, R., Laubenstein, L., Morales, J. O., Bellman, P., Yangco, B., ... & Shepard, K. V. (1995). Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *Journal of pain and symptom management*, 10(2), 89-97.

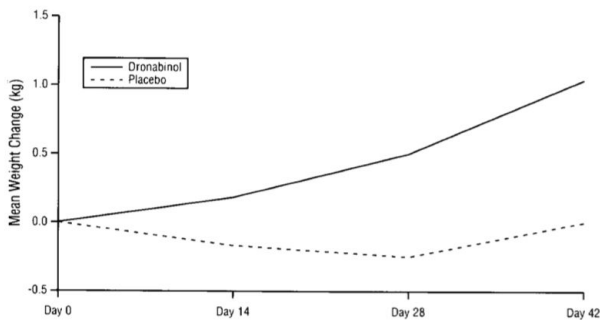


Figure 25- Mean weight change in dronabinol and placebo patients.

Source: Beal, J. E., Olson, R., Laubenstein, L., Morales, J. O., Bellman, P., Yangco, B., ... & Shepard, K. V. (1995). Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *Journal of pain and symptom management*, 10(2), 89-97.

In another study done by Foltin et al. (1988) 6 volunteers were randomized to smoke either 2 cigarettes with THC or a placebo daily (13 days total). All behaviors including food intake and social activities were recorded since the volunteers were living in a residential

laboratory. Smoking active THC increased daily caloric intake by 40%, due to increased number of snack food consumption such as candy bars and potato chips (Fotlin et al., 1988). Today, the consumption of snack foods after THC intake is referred to as the “munchies.”

It is blatant that medications are needed to prevent tissue loss that occurs from AIDS wasting syndrome. Current studies are being done using anabolic compounds such as testosterone and/or growth hormone to increase lean tissue body mass (Joy and Mack, 2000). Marijuana does not treat cachexia, however, it has been proven useful in increasing appetite and weight, as well as decreasing nausea in patients who are using ART drugs.

Peripheral neuropathy (weakness/numbness and pain from nerve damage) affects 10-30% of patients with AIDS, with the most complaints of pain in the feet/soles (Cornblath and McArthur, 1988). Sensory neuropathy in HIV cause appears to be a distal axonal degeneration of sensory neurons with late manifestation of HIV infection (Cornblath and McArthur, 1988). While some AIDS patients report that neuropathic pain can be relieved by marijuana consumption, no clinical studies have confirmed the claims (Joy and Mack, 2000).

Furthermore, HIV-1 has a tremendous effect on mood. For many people, anxiety and depression are common after an HIV diagnosis. Although it is near impossible to distinguish the treatment of anxiety or depression and the pursuit of a “high,” many patients who began using marijuana to relieve physical symptoms appreciate the psychological “lift” it provides (Joy and Mack, 2000).

B. MEDICINAL MARIJUANA TODAY

In the USA today, there are 33 states (and DC) that have legalized medicinal marijuana use. In Florida, an individual can register for a medicinal marijuana card if a doctor certifies that

the individual suffers from one or more of the following: Cancer, Epilepsy, Glaucoma, HIV/AIDS, Crohn's disease, Parkinson's disease, Multiple sclerosis (MS), Medical conditions of the same kind or class as or comparable to those above, Post-traumatic stress disorder (PTSD), Amyotrophic lateral sclerosis (ALS), A terminal condition diagnosed by a physician other than the qualified physician issuing the physician certification, and/or a chronic nonmalignant pain caused by a qualifying medical condition or that originates from a qualifying medical condition and persists beyond the usual course of that qualifying medical condition (Marijuana Policy Project, n.d).

It's important to note that everyone responds differently when using marijuana. Where some people feel calm, happy, even euphoric, some feel an increase in anxiety, dizziness, and disconnection from reality (Joy and Mack, 2000). This is why it is important to know which cannabinoids at which ratio are best suitable for the individual. The two most known cannabinoids are THC and cannabidiol (CBD).

CBD is used by patients who do not want to experience the psychoactive symptoms of THC. Different strains of marijuana have different amounts of CBD and THC. It is extremely important to use what works best for the individual.

C. INTERVIEW

Aside from scholarly studies, peer-reviewed journals, textbooks, etc. It's extremely important to hear the efficacy of therapies from those who have experienced them. In this interview, I converse with Jim Pickett, Senior Director of Prevention Advocacy at the AIDS Foundation Chicago.

1. How long ago were you diagnosed with HIV?
I was diagnosed in 1995.

2. Since being diagnosed, have you experienced
 - a. pain (neuropathy)? NO
 - b. unwanted weight loss? NO
 - c. nausea? YES
 - d. loss of appetite? YES
 - e. anxiety and/or depression YES?

3. How many medications were you prescribed in 1995 and do you remember what their functions were? The protocol at the call was to watch people's T cell count, and as their T cell level got low, then start them on medication; for the first 2 years I was not on medication at all. In 1997 I got involved in a clinical trial for a protease inhibitor.

4. Did you experience any side effects when taking these medications? If so, what were the side effects?

When I got involved in the protease clinical trial is when I started having the side effects. The anxiety and depression was more about the HIV diagnosis in general; but nausea, loss of appetite, diarrhea, lots of gastrointestinal upset that was a direct result of the protease inhibitor I was taking. They were very hard to take, I was on them for a couple years and I had nausea every day. They were very large, huge, and there were 16 of them. Timing was also an issue, you had to take them exactly when to take them, you could not really miss by even an hour. I took 8 in the morning and 8 at night plus all the other drugs. Some pills had to be refrigerated, some couldn't be refrigerated, some required food in your system, some required an empty stomach. I was taking well over 20 pills a day and 16 of them were horse pills, and they made you feel horrible. I was having a pavlovian response even before taking them, just looking at them made me feel like I was going to throw up because they made me feel so terrible. Life was completely scheduled around your medications.

5. How long after you were diagnosed did you pursue using marijuana to treat yourself and what led you to do so?

I was a recreational pot user forever, and I was using pot at the time recreationally. Medical marijuana in the 90's was like "no, no one would ever agree to that," and it seemed like a joke. I made the connection that it could be used, just a small puff before I took them and I would feel so much better.

6. What changed after you began using marijuana, symptom-wise?

I was able to get my medications down and I would be able to manage the nausea. My experience proved that marijuana as a medicine is a real thing, it's not a joke. It's helping

me stay on my meds and it's helping me get through my day because there's nothing worse than feeling nauseous all the time and you don't feel like eating. The stomach calmed my stomach down so I wasn't in such distress all the time. I could eat and tolerate the medication.

7. How much medical marijuana do you consume at a time and when would you smoke?
I smoked the old fashioned way, the leaf through a little "one-hitter pipe" that I would pack up and it was just one puff to feel immediate relief of the nausea and anticipated nausea right before taking the medication. It really was just a little bit, there was no need to get "toasted." It was amazing.
8. How do you think taking medicinal marijuana had an affect on your HIV prognosis?
I wouldn't have been able to make it if I never figured it out. I could definitely see myself not being able to maintain the regimen and maintain resistance to a possible AIDS diagnosis so it really was incredibly important to have that tool because it's a very schizophrenic state to be in. After starting on the trial I immediately started seeing the numbers, T cells immediately went up, and my markers of health were great. "The drugs were working this is fantastic, HIV is being suppressed, but I felt like hell." I've been positive since 1995, but those 2 years were the worst years of my life.
9. Do you believe that because you began using marijuana that you were able to avoid prolonging the disease to AIDs?
Definitely. It maintained my health. I never ever got AIDS. I never had my T cell count to be super dangerous because I was able to take my medications. It's a combination of things, I had access to care and had doctors who were really embedded in the HIV epidemic since the beginning. However, in terms of physically being able to take my medication those first few years before switching to other drugs, marijuana was absolutely critical. I can say that without a doubt and I'm really grateful that I just figured it out.
10. And are there particular strands that you prefer, and why? I like the ones that are more upbeat. I don't like ones that mellow you out and put you to sleep.
11. Currently, how many medications do you take for HIV-1? Side effects?
I am treatment experienced so my doctor had to make an interesting combination. I am currently taking three medications: etravirine (non-nucleoside reverse transcriptase inhibitor), Selzentry (CCR5 antagonist), and Tivicay (integrase inhibitor). I don't really have any noticeable side effects. My stomach was never the same after the protease

inhibitor drug but it's tolerable. I'm happy with it, my viral load remains suppressed to undetectable levels. Currently, my T cell count is within a normal range.

12. What are your thoughts on modifying your genome to prevent HIV-1 proliferation in your body?

I think it's really interesting! I'm all about new technology and research, especially things that can suppress HIV and give people a better quality of life.

13. What are your thoughts on receiving a bone marrow transplant or a stem cell transplant (adult or embryonic) to replace your hematopoietic system with blood cells immune to HIV?

I think a bone marrow transplant is way too invasive and scary and it's way too much. I appreciate what we've learned, but it's at a huge cost physically and in any way. I would never be interested in anything that intense. If delivery was not through BMT there would still be a lot of things I need to weigh, quality of life being the number one thing. I would have to do a risk benefit of life to consider it.

9. PREVENTATIVE MEASURES

A. PRE- EXPOSURE PROPHYLAXIS (PREP)

People who do not have HIV but are at high risk of getting HIV (either through blood or semen) take HIV medicine (PrEP) daily to reduce their chances of contracting HIV. Currently, the FDA has approved 2 medications for HIV PrEP, Truvada and Descovy. PrEP reduces the risk of getting HIV from sex by about 99% if 7 doses are taken per week (Anderson et al., 2012). The CDC states that among people who inject drugs, PrEP reduces HIV risk by at least 74% when taken daily (Pre-Exposure prophylaxis, 2019). Truvada and Descovy are combination medicines containing tenofovir and emtricitabine (both HIV nucleoside reverse transcriptase inhibitors). Its important to note that that while Truvada is recommended to prevent HIV for all people at risk (sex and needle use), Descovy for PrEP is recommended to prevent HIV through sex excluding

receptive vaginal sex (Pre-Exposure prophylaxis, 2019). Long term toxicity is still to be determined although nausea has been noted in some patients taking HIV PrEP.

B. HIV VACCINE

In 2016, the National Institute of Health (NIH) and Partners launched the first HIV vaccine efficacy trial in 7 years. The study, HVTN-702 (Uhambo), enrolled 5,400 HIV-negative volunteers at 14 sites across South Africa (First New HIV Vaccine Efficacy Study in Seven Years Has Begun, 2016). The study was targeted to prevent HIV infection in both men and women. The HVTN 702 vaccine regimen consisted of two experimental vaccines. The first is a canarypox vector-based vaccine (CPV), (ALVAC-HIV) that expresses gene products without viral replication to prevent infection; the second is a two component gp120 protein subunit vaccine with adjuvant to enhance the body's immune response to the vaccine (First New HIV Vaccine Efficacy Study in Seven Years Has Begun, 2016). Both vaccines were modified from the landmark RV144 clinical trial in Thailand from 2009 to be specific to HIV subtype C (most common in the area). The study volunteers were randomized to either receive the vaccine or a placebo, all of which will receive a total of five injections over one year (2016).

In February of 2020, the NIH discontinued HVTN-702 vaccinations because it was found to not prevent HIV. The HVTN study actually received 6 injections over 18 months (Experimental HIV Vaccine Regimen Ineffective in Preventing HIV, 2020). In January 2020 the Data and Safety Monitoring Board (DSMB) examined the data. 2,694 participants received the vaccine regimen and 2,689 received the placebo; after 60% of participants had been in the study for more than 18 months it was found that 129 HIV infections occurred in the vaccine recipients

and 123 HIV infections occurred in those who received the placebo (2020). Thus, the DSMB concluded inefficacy.

In 2017, the National Institute of Health and Partners began a HIV vaccine efficacy study that was targeted to prevent HIV acquisition among women. The study, Imbokodo, tests whether an experimental vaccine prevents HIV among 2,600 HIV-negative women in 5 sub-Saharan African countries (NIH and Partners Launch HIV Vaccine Efficacy Study, 2017). Of 1.8 million new HIV infections worldwide in 2016, 43 percent were in eastern and southern Africa (girls and women were disproportionately affected, near 60% of total) (NIH and Partners Launch HIV Vaccine Efficacy Study, 2017). The vaccine was different from that of HVTN-702 as it was modified for better efficiency and to target a wide variety of global HIV strains based on “mosaic” immunogens. The study volunteers are randomized to either receive the vaccine or a placebo, all of which will receive a total of four injections over one year; this includes 4 doses of the quadrivalent mosaic vaccine as well as a combination on the last 2 doses with doses of HIV protein clade C gp120 and aluminum phosphate adjuvant to boost immune response (2017). Participants will be followed for two years and results should be announced later this year. In preclinical studies, regimens with “mosaic-immunogen” vaccines protected monkeys against infection with an HIV-like virus (2017). Results are expected in 2021.

Similarly, in 2019, the National Institute of Health and Partners announced a phase 3 HIV efficacy trial in multiple sites in North America, South America, and Europe (clade B HIV is most common) (NIH and partners to launch HIV vaccine efficacy trial in the Americas and Europe, 2019). The study, HPX3002/HVTN 706/ Mosaico is targeted to prevent HIV acquisition among men who have sex with men and transgender people (2019). The study will enroll 3,800

HIV-negative men and transgender people age 18-60. In the US, gay/bisexual men account for $\frac{2}{3}$ of new HIV diagnoses and about 15% of transgender women have HIV. All participants were offered PrEP and were randomized to receive the experimental vaccine or a placebo. Vaccines are to be given four times a year and contain adenovirus (AD26) to deliver four mosaic immunogens with the last two doses also containing doses of mosaic gp120 (different from Imbokodo) and clade C gp120 proteins with aluminum phosphate (NIH and partners to launch HIV vaccine efficacy trial in the Americas and Europe, 2019).

10. FUTURE OUTLOOKS

Human Immunodeficiency Virus 1 was chosen for this thesis because it's evolutionary intelligence and beauty. A virus is not living and therefore is only as good as its hosts. Many people do not even know they have HIV until years after infection, since at first it only appears as common flu-like symptoms or bacterial infections. HIV has evolved to keep its hosts and therefore themselves alive for a very long time compared to other viruses.

HIV has so far defeated all attempts to completely eradicate it from the host's genome. HIV confounds the immune system in various ways. First and foremost it's target, helper T cells. T cells (both helper and killer) are recruited to fight millions of viral particles throughout the course of one's life, over the course of HIV infection, CD4 T cells deplete, and individuals are no longer able to mount an effective immune response (dependent on treatment). Those left untreated succumb to opportunistic infection but almost a decade after infection. Perhaps the most evolved tactics of HIV are (1) reverse transcriptase's high mutation rate that can cause viral diversity in a single person (2) double receptor binding of gp120/gp41 complex and a

chemokine receptor produces non-neutralizing antibodies that elicit non-neutralizing antibodies

(3) HIV carries host glycoproteins deemed “safe” from the immune system.

For those who are at high risk of exposure, PrEP should be used to prevent infection. An HIV vaccine is also underway. If unable to prevent, early detection is crucial with HIV detection. HIV is detectable about 3 weeks after infection. It is extremely important to be tested for HIV if there is probable cause of infection. 3 weeks is normally enough time for your body to build an immune response. However, early testing can render a false negative, so testing again after a week or two from the first test is the safest option.

ART drugs have so far been successful in suppressing viral loads to untransmissible levels. However, as noted from the interview, many ART drugs have undesirable side effects that can often cause one to veer off their medication regime. Medicinal marijuana has proven to help ease the conditioned responses of the drugs as well as the effects of the virus itself. Pairing ART drugs with medicinal marijuana can prove to be extremely beneficial. This combination is best for now until genome editing and stem cell treatment can be applied for HIV. Furthermore, the combination can be used for those who are against gene editing/cytotherapy for any reason.

Aside from ART drugs, gene silencing has proven to knock out different HIV proviral sequences that stop HIV proliferation *in vitro*. If HIV infection is caught early, there is less chance of HIV diversity in a single person, making the gRNA most effective. The issue using RNAi, is that although transcription cannot occur for viral products to be made, a cell will forever divide with the integrated provirus in its genome. The answer to this process is CRISPR/Cas9 cutting HIV proviral segments from the host's genome. Over time, cells will divide without the HIV until the CD4 T cells and all other cells infected are replaced. The ethical

concerns about CRISPR/CAS 9 are not to be undermined. It's also hard to imagine what the cost of this therapy would be. However, it's impossible to deny its ability to cure hundreds of what were once thought to be incurable diseases. Genome modification definitely needs an extreme amount of regulation, but this biotechnology will find its way into common practices in due time.

Replacing one's hematopoietic system with HIV resistant blood cells (via homozygous CCR5 delta 32 deletion) is the most effective practice in eradicating HIV. If a long-living homozygous CCR5delta32 donor stem cell line can be made, HIV can be wiped out of thousands of people in a short amount of time. Using this method is extremely beneficial in those who have been diagnosed with HIV for many years because it disregards HIV's high rate of mutagenicity. Pairing stem cell treatments with gene editing can also be at high benefit. Making stem hematopoietic cells immune to HIV can prove to be at more benefit than making NK cells prone to killing HIV infected cells since HIV has many ways to evade immune system response.

PrEP is very effective in preventing HIV infection. Vaccine trials are fully underway that carry various global strains. The best prevention with any sexually transmitted infection is abstinence. However, if infected, one can live a long, happy, and healthy life with HIV. ART drugs have evolutionized from 20 pills a day to possibly just one injection a month. However, it's not to say HIV cannot mutate and become resistant to these drugs, or that the toxicity from these drugs may not cause other complications later on. Plus, the fact of being HIV positive is a hard burden for many to carry because it is still very stigmatized. The solution is completely eradicating HIV either by CRISPR/CAS 9 genome modification or replacing the hematopoietic system. These methods have proven to be successful in studies explained in this thesis (either

in-vitro OR *in-vivo*). Despite the need for heavy regulation, it is safe to say that HIV infection will soon be a curable disease with these biotechnological advances.

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