Cancer Treatment: Treatment of Leukemia using HIV as a Viral Vector

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Abstract

In this article, we will examine a novel cancer treatment approach that involves the use of viral therapy. The main idea of the article is the use of HIV (which in normal circumstances targets T-cells) as a vector to transfer genetic information to a patient. HIV is the most common vector for the transfer of genetic information into a malignant cell, and it is also the most dangerous. These genetic components will assist the patient's T-cells in recognizing and damaging the malignant cells in his or her body. It may seem far-fetched to think about using a virus to treat cancer, yet it is a proven fact. When it comes to cancer treatment, HIV has proved its potential to deliver precise genetic information to the nucleus. This article will discuss the treatment's mechanism, the rationale for using HIV, specifically as a vector, and the CAR-T therapy, among other topics. Leukemia is the case of study of this article. Aside from that, it provides necessary foundation knowledge regarding HIV's behavior and binding mechanism as well as concerning viral vectors and CAR-T therapy.

I. Introduction

There were various types of leukemia therapy available many years ago. These therapies differ in their interaction with or targeting of cancerous cells. The kind of leukemia, the patient's age and health state, and whether or not the leukemia cells have moved to the cerebrospinal fluid will all influence treatment. Chemotherapy, for example, is the most common method of treatment for leukemia. Chemicals are used in this medication therapy to destroy leukemia cells. As well as being given orally through pills, chemotherapy can also be administered intravenously through a catheter or intravenous line. Another treatment used is biological therapies that assist the immune system in identifying and attacking aberrant cells. Antibodies, tumor vaccines, and cytokines are examples of biological treatments for cancer. However, instead of killing all quickly developing cells, targeted treatments are preferred to use. They interfere with a specific feature or function of a cancer cell. As a result, the targeted treatment causes less harm to normal cells than chemotherapy. Moreover, radiation treatment is another option for curing leukemia. It targets cancer cells with high-energy radiation. In addition to treating leukemia that has progressed to the brain, radiation therapy can also be used to treat leukemia that has collected in the spleen or other organs. There are other treatment approaches for leukemia, but these are the most important ones. Patients might get either one treatment or several medications depending on the kind and the severity of leukemia. In order to deal with leukemia, our research concerns on identifying leukemia and its types. Leukemia, cancer of the blood cells, affects myeloid and lymphoid in the bone marrow. Unlike other sorts of cancers that cause tumors with harmful effects in various parts of the body, leukemia cells travel freely in the bloodstream. Their harm, however, lies in their

dysfunctionality. A huge amount of useless blood cells and platelets multiply constantly in the bone marrow, clogging it up and decreasing normal blood cells.

The four main types of leukemia depend on which blood cell type they infect (myeloid & lymphoid), and the time of infection in the life-cycle of the blood cell (acute & chronic). Acute Myeloblastic Leukemia occurs in myeloblasts, marrow stem cells that specialize in RBCs, WBCs, and platelets. Acute Lymphoblastic Leukemia occurs in lymphoblasts, that specialize in T-cells, B-cells, and natural killer cells. The infected blasts lose their ability to mature and remain as useless oversized cells. Because of their size and number, they easily clog tissues upon infiltrating them, causing hepatomegaly, splenomegaly, pain in the bones, and others. A decrease in the number of normal cells, on the other hand, leads to anemia, frequent infections, hemorrhages, etc. Our approach is to use HIV as a based vector to treat cells. It might seem as madness to use a virus to treat cancer! It is really science madness. However, our findings have ensured that the there is a capability to use HIV as a treatment for leukemia.

II. HIV

HIV-1 and HIV-2 are members of the Retroviruses family, under the genus Lentiviruses. HIV (human immunodeficiency virus) is a virus that affects the immune system of the body. It is made up of two strands of RNA, 15 different types of viral proteins, and a few proteins from the previous host cell it infected, all encased in a lipid bilayer membrane. These chemicals, when combined, infect a kind of white blood cell in the body's immune system known as a T-helper cell (also called a CD4 cell). These important cells keep us healthy by defending us against infections and illnesses. From the earliest steps of viral attachment through the ultimate process of budding, each molecule in the virus plays a function in this process. HIV is incapable of reproducing on its own. Instead, the virus binds to and merges with a T-helper cell. It then takes over the cell's DNA, replicates itself within the cell, and eventually releases more HIV into the bloodstream. HIV is important in our study since it is the lentiviral vector that attaches to T-Cells and delivers the anti-leukemia transgene.

HIV structure

HIV is made up of two fundamental components: a

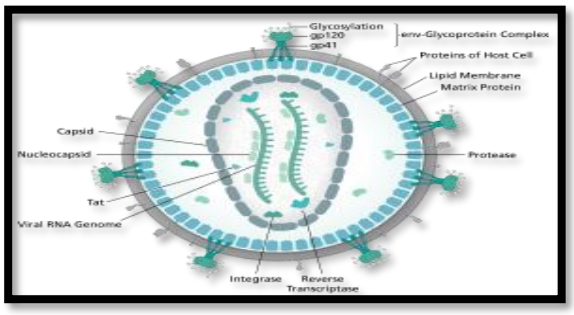

Figure 1 shows HIV structure.

ribonucleic acid (RNA) core called the genome and a protein component called the capsid that covers the genome. The genome contains the virus's genetic information, whereas the capsid gives the viral shape and protects the genome. The HIV genome is made up of three main genes: group specific antigens (Ags) or capsid proteins (gag); polymerase gene proteins (pol), which include reverse transcriptase, protease, and integrase enzymes; and envelope glycoproteins (env) [2]. (As shown in figure 1). The capsid is made up of capsomeres, which are subunits. As of today, the HIV genome is made up of nine genes, which code for a total of fifteen virus-encoding proteins. Some essential structural proteins are encoded by the genes of all retroviruses, including HIV. A number of non-structural ("accessory") HIV genes are also found. We've referred to them as Gag, or group specific antigen, viral enzymes (Pol, polymerase), and virion environment glycoproteins (Ve) when they're generated as viral polyproteins (envelope) [1]. This is the virus's general structure. It has a huge influence on its binding method and how it interacts with cells, which we will discuss in the next section.

Mechanism of HIV binding

Virions must bind to the target cell, which is done by the viral envelope (Env) protein or host cell membrane proteins integrated into the virion via any of a variety of different cell attachment factors. Attachment can be generic, with Env engaging with negatively charged cell-surface heparan sulphate proteoglycans, or more selective, with Env binding with 47 integrin or pattern recognition receptors such as dendritic cell–specific intercellular adhesion molecular 3-grabbing non-integrin (DC-SIGN) (as shown in figure 2). HIV attachment to the host cell by any of these parameters is expected to bring Env into close proximity with the viral receptor CD4 and coreceptor, boosting infection efficiency [3]. As result of HIV hides from the body's immune system in CD4 cells, it targets them via the mechanisms described above.

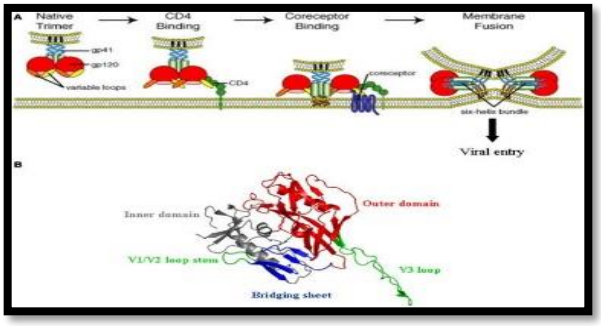

Figure 2 shows Mechanism of HIV

III. CAR-T Therapy & leukemia

Chimeric antigen receptors are specifically engineered to fit a certain antigen such that it enables the T-cells to latch onto the targeted antigen. Consequently, for a given disease, CAR's target antigen should behighly expressed in most cells/viruses that cause the disease while not having much correspondence to normal tissues, saving them from severe damage [4].

CAR T-cell therapy has been most commonly used in treating hematological cancers, especially in acute and chronic B-cell leukemia. The targeted antigen in B-cells is the CD19 molecule, a transmembrane protein that is the most expressed in all B-cells, making it a biomarker for them [2]. Recent treatment trials observed a 70–90% complete remission upon infusion of CD19 CAR T-cells, presenting the efficacy and feasibility of the therapy [5].

On the other hand, 10–20% of ALL patients have a $CD19^-$ relapse after treatment; this can be attributed to downregulation of the CD19 antigen and mutations that occur to the gene, leading the tumor to escape from the targeted detection [6]. Possible solutions are infusing a different CAR target;

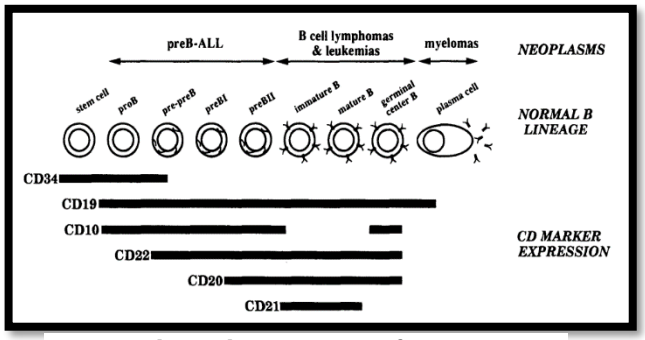

Figure 3. shows the expression of receptors in B-cells.

infusion by CD22 targeted CAR T-cells has recently shown positive results on relapsed $CD19^-$ patients.

Theoretically, applying the therapy on two tumor indicators could lengthen the duration of remission. Dual-signaling CAR-T and tandem CAR-T are two innovative techniques that have been developed. Dual signaling CAR-T cells are made up of two different CAR molecules with two different binding domains that target two different proteins of the same tumor. Tandem CAR-T cells, on the other hand, have one CAR molecule that expresses two different binding genes at the same time. CAR-T cells that target other B cell marker antigens, such as CD20 and CD22, are thus regarded to be good targets for dual or tandem CAR-T therapy for $CD19^-$ relapses and have shown positive effects [7] (as seen in figure.3). However, whether the therapy would cause an antigen loss simultaneously for both genes is still unclear. Going beyond B-cell leukemia/lymphoma (diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, high grade B-cell lymphoma, transformed follicular lymphoma, and mantle cell lymphoma.), CAR-T therapy has also been implemented on multiple myeloma patients [8] and solid tumor solutions are being actively researched [9].

As CAR T-cell therapy has achieved remarkable efficacy in the treatment of hematological malignancies, researchers got inspired to expand the application of CAR-T-cell therapy to other medical conditions like infectious diseases, autoimmune diseases, senescence-associated pathologies, etc.

CAR T-cell therapy for HIV infections

According to UNAID: Acquired immunodeficiency syndrome (AIDS), a medical condition caused by the HIV virus, has caused about 36 million deaths since it was discovered in 1980 and has a death count of 680,000 in 2020 a reduction by 64% since the peak in 2004 and by 47% since 2010. 37.7 million People are currently living with AIDS.

Antiretroviral therapy is the standard treatment for AIDS at the moment. The plasma HIV viral load could be significantly lowered or perhaps undetectable if properly applied. Despite success, new information suggests that cryptic viral replication still exists, immunological dysfunction is common during treatment, and HIV could resurface after years of undetectable viremia. Furthermore, antiretroviral therapy's side effects are considered to increase the chance of non-AIDS mortality. As a result, the current therapeutic techniques' efficacy is far from sufficient, and led to research of CAR T-cell therapy for HIV infections.

Immunotherapeutic methods to treat HIV infection have been hindered by features peculiar to HIV infection, such as the high mutation rate of reverse transcriptase, which allows for the rapid generation of immune escape mutated variants and viremia recurrence.

To destroy HIV-infected cells, first-generation anti-HIV CAR methods used the CD4 receptor's extracellular region as the targeting domain, along with the CD3ζT cell signaling domain. Later research indicated that CD4-based CARs make gene-modified T cells vulnerable to HIV infection [9]. Several ways to improve HIV-specific CAR-T cells have been tested to address this constraint, including the construction of tandem CAR-T cells, or CAR-T cells expressing a CD4 CAR in combination with either a gp41-derived fusion inhibitor or CCR5 ablation. Anti-HIV CARs have also been reengineered with 4-1BB or CD28 stimulatory signaling patterns to improve their in-body persistence and potency when used in combination with soluble broadly neutralizing antibodies (bNAbs) that identify nonredundant gp120/gp41 antigen epitopes [10].

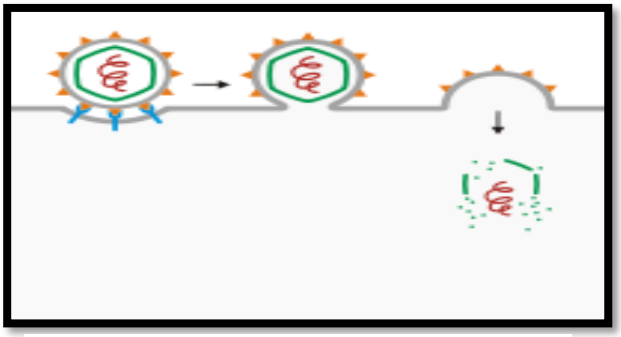

Figure 4 shows the virus introducing its gene to the host cell.

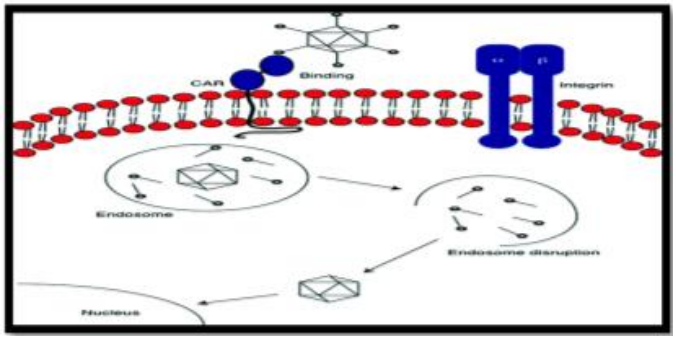

Figure 5 represents adenoviral infection.

IV. Viral vectors

Viral vectors are tools are designed to deliver therapeutic genes into the cell. Viruses are used in this process to transport nucleic acid into the genetic makeup of the cell. Viruses naturally evolved vehicles that transfer their gene to the host cell. This ability makes the viruses best suited for this process. The ligand, which is a molecule of the virus has the capability to bind to proteins. Concerning the virus, the ligand is a protein in the outer coat of the virus that binds with the receptor protein of the cell. Each cell type has a different receptor protein. Therefore, specific viruses can be used to deliver the desired gene to the target cell type. Once the ligand of the virus attaches to the receptor protein, the virus

introduces its gene DNA or RNA to the host cell (as shown in figure 4).

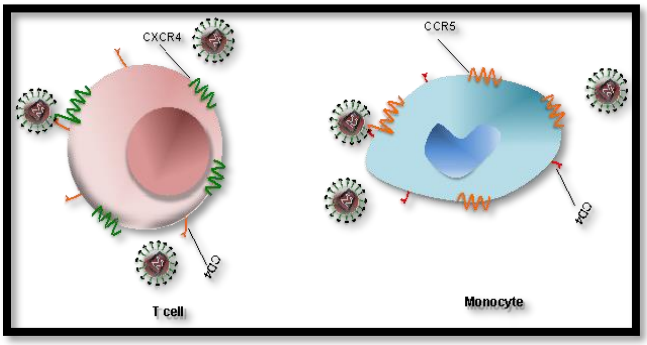

Figure 6 shows the HIV infects monocytes by interacting with the CCR5 co-receptor

Types of viral vectors and their mechanism

Specific viruses are used to transfer genetic materials to specific cell types These include adenoviruses, retroviruses, poxviruses, adeno-associated viruses, baculoviruses, and herpes simplex viruses. Now, we will explain some of these viral vectors. First, the adenoviruses species are the most effective for creating a viral vector for the use of gene therapy. The globular knob domain of the viral capsid has a high affinity for adenovirus receptors (CAR). The CAR is found on a variety of cells throughout the human body. The virus-host cell that affinity between the fibrous knob and the CAR is heightened by the interaction of the penton base protein with secondary cellular receptors. Then, the virus introduces through the cell membrane via receptor receptor-mediated endocytosis. After that, the genome inserts the protein capsid and makes its way into the host cell nucleus (as shown in figure 5).

HIV as a viral vector

The vector used in the treatment discussed in this paper is Human Immunodeficiency Virus (HIV). HIV in normal attacks the immune system especially the T-cells (a type of white blood cell that is responsible for killing foreign bodies such as viruses, bacteria, and cancer cells). HIV infects T cells via high-affinity interaction between the virion envelope glycoprotein (gp120) and the CD4 molecule. The infection of T cells is assisted by the T-cell co-receptor called CXCR4 while HIV infects monocytes by interacting with the CCR5 co-receptor (as shown in figure 6). The mechanism of using HIV as a vector is inserting the gene material we want to transfer to the t-cell. The RNA is removed to make the virus safe for the patient.

V. The mechanism of the treatment

In this section, we explain why HIV is used in this process. In addition, we elaborate on the process of using HIV as leukemia treatment

i. Why HIV

First, the normality of HIV that targets the T-cells. HIV directly infects T cells via high-affinity interaction between the virion envelope glycoprotein (gp120) and the CD4 molecule. The infection of T cells is assisted by the T-cell co-receptor called CXCR4 while HIV infects monocytes by interacting with the CCR5 co-receptor. Later than, the HIV gene transfers to the nucleus of the T-cell. Therefore, using HIV will be efficient to transfer the genetic material to the T-cell. Second, HIV is better suited to use as a vector. This is because Lentivirus-based vectors can infect non-dividing and slowly dividing cells.

ii. Genetic Materials

First, a sample is taken from the patient immune cells. Then, the gene for a special receptor that binds to a specific protein on the patient's cancer cells is added to the T-cell of the patient. This receptor is called CAR T-cells. CAR T-cells are not normally found in the immune system. The protein finds in the body of foreign bodies helps the immune system to recognize these bodies. Each protein has a particular receptor that can bind to it. These proteins are called antigens. In other words, we can describe the relationship between antigens and immune receptors is like a lock and key. Just as a lock can only be opened with the right key, each foreign antigen has a unique immune receptor that binds to it.

Since different cancers have different antigens, each CAR is made for specific cancer's antigen. For instance, in certain kinds of leukemia, the cancer cells have an antigen called CD19. The CAR T-cell therapies in order to treat these cancers are made to attach to the CD19 antigen and will not work for cancer that does not have the CD19 antigen.

iii. Making the CAR T cells

After the white cells are removed, the T cells are separated and altered by adding the gene for the specific chimeric antigen receptor (CAR). This makes them CAR -T. Then, these cells are grown and multiplied in the lab. It can take several weeks to make the large number of CAR-T needed for this therapy.

iv. Preparing HIV and Infection

HIV is in simplest terms a vector. By taking out all the viral RNA inside, HIV becomes harmless. HIV is then exposed to CAR-T cell and injects back to the patient. HIV start to target both the cancerous and not cancerous B-cells (lymphocytes that are responsible for making antigens in the body). Once the CAR -T bind with B cells, they start to increase in number and can help destroy even more cancer cells. T Cells then multiply and create a memory in the body to kill any future cancerous B-cells, preventing any type of leukemic cancer to form.

VI. Conclusion

Viruses have positive aspects as they have negative aspects. We can use viruses in the treatment of deadly diseases such as Leukemia cancer (a type of white blood cells cancer). By new technologies such as viral thereby, we can use these viruses to introduce specific genetic materials to the human body. The viruses play the role of transportation HIV in normal target the T-cells (type of white blood cells). Therefore, HIV can be used to transfer the genetic material that the T-cells need to fight against the cancerous cells in the immune system. In our case of study, we are working on leukemia disease. In this case, the T-cells themselves are cancerous. To treat this type of cancer we need certain receptor that make the T-cell able to recognize the cancer cell by binding to it. This receptor called CAR-T. First, the T-cells are taken from the patient. Then, the CAR-T receptor is added to the T-cells in the laboratory. Finally, the modified T-cells exposed to HIV. After that the HIV is introduced to the patient. The modified T-cells become able to recognize the cancerous cell and fight them. In addition, T -Cells then multiply and create a memory in the body to kill any future cancerous B-cells, preventing any type of leukemic cancer to form. There are future fields of research and development that we recommend to be done. To begin, since the precise sequence of genetic material is not unknown, attempts must be made to ascertain it. Additionally, CAR-T treatment requires further development before it can be used on human cells. Moreover, because the method of HIV insertion is somehow vague, experiments on mice must be conducted prior to human use. Also, we recommend using other viral vectors like adenoviruses and herpes simplex virus to be used in other therapies. Overall, viral vectors appear to be the key to curing many diseases. Viral vectors will be used in several therapeutics in the next years.

VII. References

[1] Andrew Peterson, Brian Seed,Genetic analysis of monoclonal antibody and HIV binding sites on the human lymphocyte antigen CD4,Cell,Volume 54, Issue 1,1988,Pages 65-72

[2] Dominic Esposito, Robert Craigie,HIV Integrase Structure and Function,Editor(s): Karl Rlaramorosch, Frederick A. Murphy, Aaron J. Shawn,Advances in Virus Research,Academic Press,Volume 52,1999,Pages 319-333,

[3] Wain-Hobson, Simon HIV genome variability in vivo, Aids: January 1989 - Volume 3 - Issue 1 - p S13-18

[4] F. Perna, S. H. Berman, R. K. Soni, J. Mansilla-Soto, J. Eyquem, M. Hamieh, R. C. Hendrickson, C. W. Brennan, and M. Sadelain, "Integrating proteomics And Transcriptomics for systematic Combinatorial chimeric Antigen RECEPTOR therapy of AML," Cancer Cell, vol. 32, no. 4, 2017.

[5]R. H. Scheuermann and E. Racila, "CD19 antigen in leukemia and lymphoma diagnosis and immunotherapy," Leukemia & Lymphoma, vol. 18, no. 5-6, pp. 385–397, 1995.

[6]K. C. Pehlivan, B. B. Duncan, and D. W. Lee, "Car-t cell therapy for acute lymphoblastic leukemia: Transforming the treatment of relapsed and refractory disease," Current Hematologic Malignancy Reports, vol. 13, no. 5, pp. 396–406, 2018.

[7] X. Xu, Q. Sun, X. Liang, Z. Chen, X. Zhang, X. Zhou, M. Li, H. Tu, Y. Liu, S. Tu, and Y. Li, "Mechanisms of relapse after cd19 car t-cell therapy for acute lymphoblastic leukemia and its prevention and treatment strategies," Frontiers in Immunology, vol. 10, 2019.

[8] T. J. Fry, N. N. Shah, R. J. Orentas, M. Stetler-Stevenson, C. M. Yuan, S. Ramakrishna, P. Wolters, S. Martin, C. Delbrook, B. Yates, H. Shalabi, T. J. Fountaine, J. F. Shern, R. G. Majzner, D. F. Stroncek, M. Sabatino, Y. Feng, D. S. Dimitrov, L. Zhang, S. Nguyen, H. Qin, B. Dropulic, D. W. Lee, and C. L. Mackall, "Cd22-targeted car t cells induce remission in b-all that is naive or resistant to cd19-targeted car immunotherapy," Nature Medicine, vol. 24, no. 1, pp. 20–28, 2017.

[9] B. Guo, M. Chen, Q. Han, F. Hui, H. Dai, W. Zhang, Y. Zhang, Y. Wang, H. Zhu, and W. Han, "Cd138-directed adoptive immunotherapy of chimeric antigen receptor (car)-modified t cells for multiple myeloma," *Journal of Cellular Immunotherapy*, vol. 2, no. 1, pp. 28–35, 2016.

[10] C. H. June, R. S. O'Connor, O. U. Kawalekar, S. Ghassemi, and M. C. Milone, "Car t cell immunotherapy for human cancer," Science, vol. 359, no. 6382, pp. 1361–1365, 2018.

[11] L. Liu, B. Patel, M. H. Ghanem, V. Bundoc, Z. Zheng, R. A. Morgan, S. A. Rosenberg, B. Dey, and E. A. Berger, "Novel cd4-based bispecific chimeric antigen receptor designed for enhanced anti-hiv potency and absence of hiv entry receptor activity," Journal of Virology, vol. 89, no. 13, pp. 6685–6694, 2015.

[12] K. Anthony-Gonda, A. Bardhi, A. Ray, N. Flerin, M. Li, W. Chen, C. Ochsenbauer, J. C. Kappes, W. Krueger, A. Worden, D. Schneider, Z. Zhu, R. Orentas, D. S. Dimitrov, H. Goldstein, and B. Dropulić, "Multispecific anti-HIV duoCAR-T cells Display broad in vitro antiviral activity and potent in vivo elimination OF HIV-infected cells in a humanized mouse model," Science Translational Medicine, vol. 11, no. 504, 2019