Viral vectors for gene delivery : precision medicine in patient care

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Introduction

The possibility of gene therapy began in the 1990's and quickly grew as a hopeful new way to treat patients suffering from debilitating diseases. Instead of treating simply the symptoms, patients would now have a means to be cured of their diseases, with hopefully fewer side effects than the current treatments in practice. For example, patients would no longer need transfusions to prevent them from becoming anemic. Another exciting possibility would be to cure cancer without the devastating effects of chemotherapy. In 1993 the first clinical trial showed great promise by helping participants who were suffering from adenosine deaminase deficiency (ADA) (Blaese, 1993). This was quickly overshadowed by the unfortunate death of Jesse Gelsinger (Stolberg, 1999) slowing the progress of viral mediated gene therapy and caused researchers to find new viral vectors with less risks associated to their use.

Retroviral (RV), lentiviral (LV), oncoretroviruses, adenoviruses (AV), adeno-associated viruses (AAV), and herpes simplex-1 (HAV-1) vectors are currently used the most often in viral-mediated gene delivery (Thomas, Ehrhardt, & Kay, 2003). When developing a viral vector the researcher must include the ability to regulate where and when the therapeutic genes will be expressed (Das et al., 2015). Such regulation has led to the use of self-inactivating (SIN) viral vectors that decrease the possibility of activating genes close to the insertion site of the therapeutic gene (Kotterman, Chalberg, & Schaffer, 2015). Breakthroughs such as SIN viral vectors have allowed researchers to take another look at the use of viral vectors in gene therapy.

Currently, cancer therapies have taken the forefront in the field of viral vector research (Ginn, Alexander, Edelstein, Abedi, & Wixon, 2013). Other clinical trials have also showed promising results with the use of a viral vector. β-thalassaemia was treated in one patient allowing him to no longer require blood transfusions (Cavazzana-Calvo et al., 2010). Two

participants with X-linked adrenoleukodystrophy (ALD) showed an increase of ALD expression up to fifty percent (Cartier et al., 2009). Participants suffering from Wiskott-Aldrich Syndrome showed an increase in immune function following treatment (Aiuti et al., 2013). One patient suffering from metachromatic leukodystrophy (MLD) was able to walk with assistance (Biffi et al., 2013). This was an accomplishment that was not seen in other patients who did not undergo treatment.

In 2008, the first commercially available viral vector treatment was released (Das et al., 2015) and the breakthrough opened again the possibility of using gene therapy in a clinical setting. An accumulation of over twenty years of clinical trials should open doors for gene therapy to be used in the clinic. Herein, we will specifically discuss the progress and use of lentiviral vectors, and their effective means for treatment in the clinic.

Literature Review

History of Gene Therapy

In 1990 a clinical trial began to treat two participants who suffered from ADA deficiency. Patients with this disease deal with recurrent opportunistic infections due to a defect in the enzyme adenosine deaminase. Without the enzyme, toxic compounds build up within immune cells, preventing them from functioning (Blaese et al., 1995). The researchers used a RV vector to transduce T cells with new ADA genes and showed a 10-fold increase from previous ADA expression levels (Blaese, 1993). A four-year observation of the initial patients revealed that both patients showed continued improvement of ADA expression (Blaese et al., 1995), which prompted researchers to begin using viral vectors on other genetic abnormalities in the hopes to find another treatment. Unfortunately, the promise of viral vector use in clinical trials was

quickly overshadowed by unforeseen side effects. In 1998 the death of Jesse Gelsinger was the first loss directly related to gene therapy (Chira et al., 2015). The cause of his death was due to the AV vector inducing a massive inflammatory response that led to disseminated intravascular coagulation, acute respiratory distress (Thomas et al., 2003), and multiorgan failure (Kotterman et al., 2015). Immune response to the viral vector has been called by some as the "Achilles heel" of gene therapy (Thomas et al., 2003). It seemed, for some time, that gene therapy would not be a feasible treatment for patients. The use of viral vectors was reassessed for clinical use, and regulations were put in place to prevent further harm.

Other reports of poor clinical outcomes continued to show up. One major side effect seen included insertional mutagenesis. It is a process where the viral vector integrates the genetic material near a proto-oncogene promoter or other site that regulates cell growth (Ginn et al., 2013). Should a gene be inserted into an area where a tumor suppressor gene is located it could cause tumor growth, or the development of leukemia (Misra, 2013) and potentially other concerns. In 2003, an article reported that two participants in a trial to treat X-linked severe combined immunodeficiency (SCID-X1) developed uncontrolled T cell proliferation (Hacein-Bey-Abina et al., 2003). In 2008, they reported that two more participants had developed the same clonal proliferation of T cells (Hacein-Bey-Abina et al., 2008). These reports brought forth some of the biggest challenges known to viral vector mediated gene delivery causing researchers to again weigh the cost verses benefit of therapeutic viral vector use. Prevention of insertional mutagenesis soon became a baseline marker in the development of "safe" viral vectors.

Development of Viral Vectors

New developments in viral vector design have increased the efficiency and safety of gene therapy. To obtain the desired results the viral vector must be able to get in to the nucleus and

deliver the therapeutic gene. This must be done without causing the known side effects of insertional mutagenesis, and the induction of massive immune responses by patients upon receiving treatment. Many approaches have been taken in developing the best-suited viral vector that would safely deliver the genetic material. An early step in the process of treatment is to decide what properties of the viral vector are suitable for the therapy. There exists two main ways that viral vectors function: viral vectors that integrate within the host genetic material, and those that do not. The integrating vectors include RV and LV, whereas non-integrating vectors include AV and AAV (Das et al., 2015). By integrating, LV vectors allow the cells to pass on the transduced genetic material to its daughter cells (George & Fogarty, 2016). This creates a permanent cure for the patients, but also opens the door to the possibility of insertional mutagenesis that must be addressed before use of these viral vectors can become the first line therapy which early researchers hoped.

Early development of AV vectors included deletions in the viral vector genome that prevented it from entering its lysogenic cycle (Chira et al., 2015). However the viral vector was still able to cause an immune response by the host as was seen in the trial with Mr. Gelsinger. Other means of safety is the development of self-inactivating viral vectors (SIN) which is accomplished by deleting regions of the 3′ located in the long terminal repeats (LTR) (Rothe, Modlich, & Schambach, 2013). The regions include the enhancer and promoter and upon transduction within the host cell will instead duplicate the 3′ LTR during reverse-transcription (Chira et al., 2015). Spatial segregation can also be used to increase the safety of the viral vector. This happens by separating helper plasmids, such as gag, pol, and env, from the carrying viral vector (Rothe et al., 2013), decreasing the likelihood of having a viral vector that can

replicate within the host. Ultimately, this leads to the use of plasmids that can generate the viruses in cell culture that can be purified for therapeutic delivery in patients.

The next step is to decide if the treatment needs to target dividing or non-dividing cells. Lentiviral vectors, which HIV falls under, have the ability to integrate within cells that are not actively dividing (Demeulemeester, De Rijck, Gijsbers, & Debyser, 2015). To do this the virus has developed an ability to attach to the nuclear pore and deliver the viral material within the intact nuclear envelope (Demeulemeester et al., 2015). This process increases the likelihood that the treatment will be delivered to the target cells that are not dividing. Typically, cancer cells are rapidly dividing, however; not always, and this method allows targeting of non-dividing cells.

The LV vector's ability to integrate within the genome of the target cell is yet another step in the development of the viral vector. LV vectors have shown a preference to integrate in regions that are actively transcribed possibly because of the availability of chromatin during transcription (Desfarges & Ciuffi, 2010). The more convincing argument is that a protein called Lens Epithelium-Derived Growth Factor/p75 (LEDGF/p75) attaches the viral package to the active regions of the genome (Demeulemeester et al., 2015). By knowing the process by which the viral vector attaches to the genome, researchers can pick which one would be most beneficial for their research. Thus, allowing more specificity in the treatment.

Hematopoietic Stem Cell Transplantation –Gene Therapy

Hematopoietic stem cell transplantation gene therapy (HSCT-GT) involves removing stem cells from the patient then exposing the cells to the viral vector allowing the desired gene to be transduced into the cells (Biffi et al., 2013). This process has been used in numerous studies as a means of a possible long-term cure. Researchers have found that myeloablative treatment used prior to transplantation has decreased transplant rejection in some instances (Cartier et al.,

2009). By using this process of gene therapy researchers have revealed possible means of treatment for patients with diseases that have a poor prognosis. One such disease is X-linked adrenoleukodystrophy (ALD), which is a degenerative disease that results in accumulation of very-long-chain fatty acids (VLCFA) that can potentially cause demyelination in the cerebral tissue (van de Beek et al., 2016). One current treatment for ALD is a transplant from a donor which does not come without risk to the patient due to the potential of developing graft versus host disease (GVHD) (Fischer, Hacein-Bey Abina, Touzot, & Cavazzana, 2015).

Donor matching has decreased GVHD development, but has also made it harder for some patients to receive treatment. For example, a study was done with two participants that were not able to receive the conventional transplantation for ALD. Blood samples were taken from the participants and their CD34+ cells were activated *ex vivo* by cytokines to induce growth and replication. The cells were then introduced to the ALD gene via HIV-1-derived LV vector, which transduced the patient's target cells. Five days following infusion, the ALD expression was measured initially showing 50% of patient (ALD-P1) and 33% of patient 2 (ALD-P2) CD34+ cells expressed the ALD protein. However, ALD expression dropped to 10% and 15% respectively. Magnetic resonance imaging (MRI) results revealed that demyelination was stabilized 16 months after treatment. Plasma levels of VLCFA decreased 39% in ALD-P1 and 38% in ALD-P2 (Cartier et al., 2009). Demyelination remained halted on follow up of patients three years after treatment (Cartier et al., 2012). These results showed that LV vector use could be a potential treatment for patients who did not qualify for the conventional treatment.

Another treatment option is the use of a self-inactivating HIV-type 1 vector to deliver the β-globin gene. Of five patients treated, the most remarkable results came from patient 2 (βg-P2) who was an 18-year-male suffering from a severe form of β-thalassemia deficiency. Following

treatment, βg-P2 no longer required back up transfusions. Twenty-one months following βg-P2's final transfusion his mean corpuscular hemoglobin (MCH) measured 28.7 pg, an increase of 9.7% when compared to others who did not receive the treatment (Cavazzana-Calvo et al., 2010). Though transfusions and iron binding medications have been used for a long time, this study showed it may be another option for patients who decide on an alternative therapy.

Wiskott-Aldrich Syndrome (WAS) is another disorder that has benefited from clinical trials using viral vectors. It is an X-linked immunodeficiency that involves frequent infections, eczema, thrombocytopenia (Ghosh, Thrasher, & Gaspar, 2015), and autoimmune disorders (Candotti, 2016). The main cause is a defect in the WAS gene, which is involved in cytoskeleton development and affects all hematological lines leading to the stated clinical presentations (Hacein-Bey Abina et al., 2015). In this trial, three participants received the treatment of HSCT-GT. Thirty months after treatment, all three participants showed great improvement in cell growth both in the bone marrow and in peripheral blood samples. The WAS protein was expressed in the T cells, natural killer cells (NK), and monocytes. NK cells showed normal cytotoxic activity in all three participants. Preexisting eczema cleared up within 12 months, and platelet count increased, and none of the participants experienced thrombocytopenia upon cessation of platelet transfusions (Aiuti et al., 2013). These results showed improvement of viral vector use when compared to earlier treatments of WAS that caused patients to develop leukemia through insertional mutagenesis.

Another study using HSCT-GT to treat patients with metachromatic leukodystrophy (MLD) showed favorable outcomes in early clinical trials. This disorder has a deficiency in the enzyme arysulfatase-A (*ARSA*) gene resulting in the accumulation of sulfatide substrates within the central nervous system and other organs. Patients with the severe form suffer with ataxia,

dysphasia, deafness, seizures, and in the worst cases death (Biffi, Lucchini, Rovelli, & Sessa, 2008). The results from this study showed that *ARSA* was present and functioning as early as one-month post HSCT-GT treatment. Hydrolysis of sulfatide was confirmed in the patients, and at 39 months, one patient was able to stand and walk with assistance (Biffi et al., 2013). Through viral vector use some patients with MLD have been able to find a means of preventing the debilitating symptoms common in the disorder.

Cancer

Researchers continue to develop means to treat patients suffering genetic disorders, but the main focus of gene therapy has been on cancer. In 2003 it was reported that 63.4 % of gene therapy trials were focused on cancer treatment (Thomas et al., 2003). Close to ten years later a study showed that 1843 trials had been accomplished, and that 64.4% of the trials still revolved around the treatment of cancer (Ginn et al., 2013). Most treatments for cancer have included the use of chimeric antigen receptors (CAR). CARs are aimed specifically at modifying immune cells to get them to recognize the cancer cells. The use of CARs began in 1992 when Zelig Eshhar and associates developed the process to modify T cells through a viral vector (Eshhar, Waks, Gross, & Schindler, 1993). The modified T cell formed what is called a CAR which contains a single chain variable fragment (scFv) that is specific to the cancer cell antigen (Turtle, Riddell, & Maloney, 2016). Upon recognition of the antigen, the T cell begins to proliferate and destroy cells containing the scFv marker (Zhang et al., 2016). Later generations of CARs have increased their antitumor efficacy by including costimulatory sequences that increase the cytokine response of activated T cells (Milone et al., 2009). The results of CARs can be seen mostly in the treatment of hematological cancers (Qin et al., 2016). This is in part because of the complexity of selecting the correct antigen to target, and the microenvironment created by the

solid tumor that suppresses the immune response (Zhang et al., 2016). Through the process of CAR participants, who are not candidates for other treatments, have now found a means for therapy.

Although CARs have seen encouraging results a common thread of side effects is seen. Most participants experience what is called a cytokine releasing syndrome (CRS) (Garfall et al., 2015). Symptoms include fevers, hypotension, coagulopathy, and capillary leakage (Turtle, Riddell, et al., 2016). In severe cases neurotoxicity has been seen encompassing seizures, and encephalopathy (Porter et al., 2015). The severity of the CRS has been proposed to be related to the tumor burden of the patient prior to treatment (Brentjens et al., 2013). CRS can usually be treated with the administration of glucocorticoids, interleukin inhibitors, and tumor necrosis factor antagonists. Some, unfortunately, have been put on mechanical ventilation, and vasoactive medication due to the severity of the CRS (Grupp et al., 2013). Even with continued monitoring the risk of death is still present.

CRS, although severe, is not the only side effect know with the use of CARs. B cell aplasia can also be seen when treating hematologic cancers. Many researchers use the CD19 receptor on B cells as a target for the CAR. This is a marker that is specific to B cells preventing the possible complication of attacking other immune cells (Oluwole & Davila, 2016), which is a known side effect due to the specific targeting of the cells. B cells are however known to be important in fighting diseases, and therefore increase the risk of infection when not present. The depletion of B cells was noted up to four years in some participants (Porter et al., 2015). These findings have helped researchers develop ways to monitor patients undergoing treatment to prevent undue harm. Like all treatments researchers must weigh the cost verses benefit of the treatment.

Chronic lymphocytic leukemia (CLL) is currently the most prevalent adult leukemia (Porter et al., 2015). CLL is caused by a clonal overproduction of B cells that affects the bone marrow, and other lymphoid tissue (Rombout, Verhasselt, & Philippe, 2016). A study was done using viral vectors on three participants suffering from CLL. At the time this article was published, one patient continued to be in remission of CLL more than 10 months with no circulating CLL cells after 6 months of treatment. Bone marrow biopsy results from participants showed decreased infiltration as early as one month after treatment (Kalos et al., 2011). Other trials with hematologic cancers have revealed similar results.

A study of 5 participants with morphological disease (MRD+) acute lymphoblastic leukemia (ALL) each became MRD- within thirty days of using viral vectors (Brentjens et al., 2013). Another study revealed an increase in lymphocytes and neutrophils within two weeks of treatment, with remission still present after nine months in one patient (Grupp et al., 2013). In 2013 a trial that included thirty participants with (ALL) showed complete remission of leukemia in twenty seven, approximately 90%, one month after transfusion. Nineteen of the patients continued to be in remission in 2014 (Maude et al., 2014). A study published this year showed twenty seven of thirty patients with ALL did not have cancer cells present on high-resolution flow cytometry following treatment using CAR (Turtle, Hanafi, et al., 2016). Each of these studies showed great results when using viral vectors for treatment.

Conclusion

Though lentiviral use in gene therapy has shown remarkable progress in the past ten years, it has not yet yielded the desired results that earlier researchers had hoped. The idea, while revolutionary, has run into many challenging obstacles. Complete organ failure in a single

patient and the development of cancer have impeded viral vector therapies. To improve the initial obstacles researchers are now using alternative viral vectors that are 'safer,' and regulations have been put in place to prevent such undesired outcomes. The development of SIN viral vectors has reduced insertional mutagenesis and decreased the risk of developing cancer. With these changes in place, researchers have been able to make progress toward the goal of use in the clinic. The studies mentioned in this review are but a few of the many ongoing clinical trials using viral vectors. The biggest breakthroughs have been with CAR, which has dominated the field of gene therapy. The results from multiple studies have shown that viral vector use may be a means of treatment for patients suffering from hematological cancers with some reports showing remission in 90% of the participants, which may be because of the negative results of CRS that is commonly seen with CAR. In most articles dealing with CAR, the researchers found that participants developed CRS with varying degrees of severity. In some studies, CRS has been severe enough to take the lives of participants. Turtle and associates had the unfortunate outcome of two patients' deaths directly related to CRS. Outcomes like this demonstrate how much work is still needed before wide-spread therapy utilizing viral vectors. With this in mind, researchers may be able to create parameters that include tumor burden within the treatment. In time, viral vectors may still be useful within the clinic and as a therapeutic in humans.

Citations

Aiuti, A., Biasco, L., Scaramuzza, S., Ferrua, F., Cicalese, M. P., Baricordi, C., . . . Naldini, L. (2013). Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. *Science, 341*(6148), 1233151. doi:10.1126/science.1233151

Biffi, A., Lucchini, G., Rovelli, A., & Sessa, M. (2008). Metachromatic leukodystrophy: An overview of current and prospective treatments. *Bone Marrow Transplantation, 42*(Suppl 2), S2-6. doi:10.1038/bmt.2008.275

Biffi, A., Montini, E., Lorioli, L., Cesani, M., Fumagalli, F., Plati, T., . . . Naldini, L. (2013). Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science, 341*(6148), 1233158. doi:10.1126/science.1233158

Blaese, R. M. (1993). Development of gene therapy for immunodeficiency: Adenosine deaminase deficiency. *Pediatric Research, 33*(1 Suppl), S49-53; discussion S53-45. doi:10.1203/00006450-199305001-00278

Blaese, R. M., Culver, K. W., Miller, A. D., Carter, C. S., Fleisher, T., Clerici, M., . . . Anderson, W. F. (1995). T lymphocyte-directed gene therapy for ADA- SCID: Initial trial results after 4 years. *Science, 270*(5235), 475-480.

Brentjens, R. J., Davila, M. L., Riviere, I., Park, J., Wang, X., Cowell, L. G., . . . Sadelain, M. (2013). CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Science Translational Medicine, 5*(177), 177ra138. doi:10.1126/scitranslmed.3005930

Candotti, F. (2016). Advances of gene therapy for primary immunodeficiencies. *F1000Research, 5*. doi:10.12688/f1000research.7512.1

Cartier, N., Hacein-Bey-Abina, S., Bartholomae, C. C., Bougneres, P., Schmidt, M., Kalle, C. V., . . . Aubourg, P. (2012). Lentiviral hematopoietic cell gene therapy for X-linked adrenoleukodystrophy. *Methods in Enzymology, 507*, 187-198. doi:10.1016/b978-0-12-386509-0.00010-7

Cartier, N., Hacein-Bey-Abina, S., Bartholomae, C. C., Veres, G., Schmidt, M., Kutschera, I., . . . Aubourg, P. (2009). Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science, 326*(5954), 818-823. doi:10.1126/science.1171242

Cavazzana-Calvo, M., Payen, E., Negre, O., Wang, G., Hehir, K., Fusil, F., . . . Leboulch, P. (2010). Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature, 467*(7313), 318-322. doi:10.1038/nature09328

Chira, S., Jackson, C. S., Oprea, I., Ozturk, F., Pepper, M. S., Diaconu, I., . . . Berindan-Neagoe, I. (2015). Progresses towards safe and efficient gene therapy vectors. *Oncotarget, 6*(31), 30675-30703. doi:10.18632/oncotarget.5169

Das, S. K., Menezes, M. E., Bhatia, S., Wang, X. Y., Emdad, L., Sarkar, D., & Fisher, P. B. (2015). Gene therapies for cancer: Strategies, challenges and successes. *Journal of Cellular Physiology, 230*(2), 259-271. doi:10.1002/jcp.24791

Demeulemeester, J., De Rijck, J., Gijsbers, R., & Debyser, Z. (2015). Retroviral integration: Site matters: Mechanisms and consequences of retroviral integration site selection. *Bioessays, 37*(11), 1202-1214. doi:10.1002/bies.201500051

Desfarges, S., & Ciuffi, A. (2010). Retroviral integration site selection. *Viruses, 2*(1), 111-130. doi:10.3390/v2010111

Eshhar, Z., Waks, T., Gross, G., & Schindler, D. G. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proceedings of the National Academy of Sciences U S A, 90*(2), 720-724.

Fischer, A., Hacein-Bey Abina, S., Touzot, F., & Cavazzana, M. (2015). Gene therapy for primary immunodeficiencies. *Clinical Genetics, 88*(6), 507-515. doi:10.1111/cge.12576

Garfall, A. L., Maus, M. V., Hwang, W. T., Lacey, S. F., Mahnke, Y. D., Melenhorst, J. J., . . . Stadtmauer, E. A. (2015). Chimeric antigen receptor T cells against CD19 for multiple myeloma. *New England Journal of Medicine, 373*(11), 1040-1047. doi:10.1056/NEJMoa1504542

George, L. A., & Fogarty, P. F. (2016). Gene therapy for hemophilia: Past, present and future. *Seminars in Hematology, 53*(1), 46-54. doi:10.1053/j.seminhematol.2015.10.002

Ghosh, S., Thrasher, A. J., & Gaspar, H. B. (2015). Gene therapy for monogenic disorders of the bone marrow. *British Journal of Haematology*. doi:10.1111/bjh.13520

Ginn, S. L., Alexander, I. E., Edelstein, M. L., Abedi, M. R., & Wixon, J. (2013). Gene therapy clinical trials worldwide to 2012 - An update. *Journal of Gene Medicine, 15*(2), 65-77. doi:10.1002/jgm.2698

Grupp, S. A., Kalos, M., Barrett, D., Aplenc, R., Porter, D. L., Rheingold, S. R., . . . June, C. H. (2013). Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *New England Journal of Medicine, 368*(16), 1509-1518. doi:10.1056/NEJMoa1215134

Hacein-Bey Abina, S., Gaspar, H. B., Blondeau, J., Caccavelli, L., Charrier, S., Buckland, K., . . . Cavazzana, M. (2015). Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. *JAMA, 313*(15), 1550-1563. doi:10.1001/jama.2015.3253

Hacein-Bey-Abina, S., Garrigue, A., Wang, G. P., Soulier, J., Lim, A., Morillon, E., . . . Cavazzana-Calvo, M. (2008). Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *Journal of Clinical Investigation, 118*(9), 3132-3142. doi:10.1172/jci35700

Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M. P., Wulffraat, N., Leboulch, P., . . . Cavazzana-Calvo, M. (2003). LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science, 302*(5644), 415-419. doi:10.1126/science.1088547

Kalos, M., Levine, B. L., Porter, D. L., Katz, S., Grupp, S. A., Bagg, A., & June, C. H. (2011). T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Science Translational Medicine, 3*(95), 95ra73. doi:10.1126/scitranslmed.3002842

Kotterman, M. A., Chalberg, T. W., & Schaffer, D. V. (2015). Viral vectors for gene therapy: Translational and clinical outlook. *Annual Review of Biomedical Engineering, 17*, 63-89. doi:10.1146/annurev-bioeng-071813-104938

Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., . . . Grupp, S. A. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *New England Journal of Medicine, 371*(16), 1507-1517. doi:10.1056/NEJMoa1407222

Milone, M. C., Fish, J. D., Carpenito, C., Carroll, R. G., Binder, G. K., Teachey, D., . . . June, C. H. (2009). Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Molecular Therapy, 17*(8), 1453-1464. doi:10.1038/mt.2009.83

Misra, S. (2013). Human gene therapy: A brief overview of the genetic revolution. *Journal of the Association of Physicians of India, 61*(2), 127-133.

Oluwole, O. O., & Davila, M. L. (2016). At the bedside: Clinical review of chimeric antigen receptor (CAR) T cell therapy for B cell malignancies. *Journal of Leukocyte Biology*. doi:10.1189/jlb.5BT1115-524R

Porter, D. L., Hwang, W. T., Frey, N. V., Lacey, S. F., Shaw, P. A., Loren, A. W., . . . June, C. H. (2015). Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Science Translational Medicine, 7*(303), 303ra139. doi:10.1126/scitranslmed.aac5415

Qin, D. Y., Huang, Y., Li, D., Wang, Y. S., Wang, W., & Wei, Y. Q. (2016). Paralleled comparison of vectors for the generation of CAR-T cells. *Anticancer Drugs*. doi:10.1097/cad.0000000000000387

Rombout, A., Verhasselt, B., & Philippe, J. (2016). Lipoprotein lipase in chronic lymphocytic leukemia: Function and prognostic implications. *European Journal of Haematology*. doi:10.1111/ejh.12789

Rothe, M., Modlich, U., & Schambach, A. (2013). Biosafety challenges for use of lentiviral vectors in gene therapy. *Current Gene Therapy, 13*(6), 453-468.

Stolberg, S. G. (1999, November 28). The biotech death of Jesse Gelsinger. *New York Times Magazine*, 136-140, 149-150. Retrieved from http://www.nytimes.com/1999/11/28/magazine/the-biotech-death-of-jesse-gelsinger.html

Thomas, C. E., Ehrhardt, A., & Kay, M. A. (2003). Progress and problems with the use of viral vectors for gene therapy. *Nature Reviews. Genetics, 4*(5), 346-358. doi:10.1038/nrg1066

Turtle, C. J., Hanafi, L. A., Berger, C., Gooley, T. A., Cherian, S., Hudecek, M., . . . Maloney, D. G. (2016). CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *Journal of Clinical Investigation, 126*(6), 2123-2138. doi:10.1172/jci85309

Turtle, C. J., Riddell, S. R., & Maloney, D. G. (2016). CD19-Targeted chimeric antigen receptor-modified T-cell immunotherapy for B-cell malignancies. *Clinical Pharmacology & Therapeutics, 100*(3), 252-258. doi:10.1002/cpt.392

van de Beek, M. C., Dijkstra, I. M., van Lenthe, H., Ofman, R., Goldhaber-Pasillas, D., Schauer, N., . . . Kemp, S. (2016). C26:0-Carnitine is a new biomarker for X-linked adrenoleukodystrophy in mice and man. *PLoS One, 11*(4), e0154597. doi:10.1371/journal.pone.0154597

Zhang, H., Ye, Z. L., Yuan, Z. G., Luo, Z. Q., Jin, H. J., & Qian, Q. J. (2016). New strategies for the treatment of solid tumors with CAR-T cells. *International Journal of Biological Science, 12*(6), 718-729. doi:10.7150/ijbs.14405

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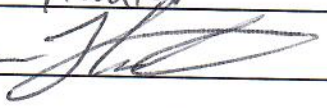
Signature: 

Department: Physician Assistant Studies College: Medicine and Life Sciences

Project Type (Circle one): **Doctoral Project** **Masters Project** **Senior Project**

Complete Title: Viral Vectors for Gene Delivery: Precision Medicine in Patient Care

Date Completed: 12/14/16 Date Approved: 12/14/16

Date Signed: 12/16/16