# How do Ethical Concerns Regarding CRISPR/Cas9 and Stem Cell Therapy Affect the Research for Volumetric Muscle Loss (VML)?

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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## Introduction

Volumetric muscle loss (VML) occurs due to a traumatic experience, commonly a war-related injury, but can happen to anyone. Injuries to civilians can include motor vehicle accidents or other similar secondary casualties. VML refers to the depletion of skeletal muscle leading to subsequent functional limitations (Grogan et al., 2011). Typically, skeletal muscle exhibits an impressive ability to regenerate upon injury. However, in the face of significant traumatic injury, the regenerative mechanism consistently proves inadequate (Aguilar et al., 2018). Without regeneration of new muscle, these patients, commonly soldiers, suffer from loss of function, and in turn, their personal lives can suffer too. As a possible treatment for VML injuries, the Christ Lab at UVA has created a Tissue-Engineered Muscle Repair construct (TEMR) involving the seeding of muscle progenitor cells (MPCs) onto a bladder acellular matrix (BAM). Subsequently, the construct undergoes incubation in a bioreactor to prepare the graft for surgical implantation (Machingal et al., 2011). After the grafts have reached the fiber alignment similar to the natural muscle fibers in the body, the bioreactor will be taken to a hospital where doctors and medical staff prepare for implantation. However, there is no cure for this ailment Studies are still being conducted to find viable treatment options for this condition. Some possible treatment options are deemed unethical, though they still have great potential to help solve complications like VML.

This research will investigate how ethical dilemmas regarding the use of genetic modification and stem cell therapies hinder clinical research for medical ailments like VML. Genetic engineering technologies like CRISPR/Cas9 could be a viable solution. CRISPR stands for clustered regularly interspaced palindromic repeats. It enables the rectification of genetic errors and the swift manipulation of gene expression in cells and organisms, with affordability,

rapidity, and relative simplicity (Redman et al., 2016). Cas9 is an endonuclease and it stands for "CRISPR-associated protein 9". This makes it possible for edits to be made within the genome. It physically creates a break in the DNA strands for edits to be made (Redman et al., 2016). However, this technology is very controversial since it can change human genomes. Another possible direction for solving VML could be the use of stem cells. Stem cells, found in both embryos and adults, are unspecialized cells that can become any cell type in the body and have the ability to renew themselves (Zakrzewski et al., 2019). The term "stem cells" encompasses a diverse array of categories that delineate their characteristics and capabilities. An example includes pluripotent stem cells which are popular due to their ability to 'rise to all of the differentiated cells in the body, such as heart muscle cells, blood cells, and nerve cells' (*Stem Cell Basics | STEM Cell Information*, n.d.). With ongoing studies, there may be many cell options that would prove beneficial for treatment. VML is detrimental for soldiers and other victims but, with the use of genetic engineering techniques and or stem cell therapies, this problem could have better clinical outcomes, and faster recovery times, and even become the solution we have been working towards.

**Significance**

As of 2024, despite how many wounded warriors and civilians there are, there is no effective treatment available for VML (Tanner et al., 2024). The interventions available include metal bracing of the injury or "patchwork" that is from the patient's skin but these are not effective as they do not solve the lack of skeletal muscle loss (Grogan et al., 2011). Based on the years 2005 to 2009 from Afghanistan and Iraq, 'seventy-seven percent of all casualties [from U.S. service members] sustained a musculoskeletal wound' (Belmont et al., 2013). Because there

is currently no substantial method for tissue regeneration of muscle loss, patients suffer from loss of function and strength. Many groups are attempting to find cures, however, there have not been any advancements. Newer technologies and techniques have been utilized in many different areas and regenerative medicine is one of them. CRISPR/Cas9 and stem cell therapy show promise for their abilities, but the ethical concerns that follow them are slowing their progress in studies. The question being examined in this paper is "How do ethical concerns regarding CRISPR/Cas9 and stem cell therapy affect research for VML?".

As science progresses rapidly, some feel excluded from discussions on its application in new technologies. Furthermore, its increasing importance often conflicts with traditional ethical beliefs, leading to ongoing challenges (Iaccarino, 2001). In areas where science interferes with natural human life, ethics takes a toll on research where the hope is to better human lives. According to the authors of a paper reviewing the ethical concerns of genetic technology, 'the most contentious issues concerning human germline modifications are the challenges to human safety and morality such as the risk of unforeseen, undesirable effects in clinical applications particularly to correct or prevent genetic diseases, matter of informed consent and the risk of exploitation for eugenics [or the improvement of the human species] ' (Shinwari et al., 2018). Furthermore, when employing stem cells for therapeutic interventions, scientists must exercise caution in light of the cell source and their potential differentiation capabilities. A source frequently used when stem cells first entered scientific research was the human embryo. This was a valuable source because they can develop into a cell type that is present in a developing fetus (Rippon & Bishop, 2004). The ethical dilemmas surrounding the human embryonic stem cell (hESC) research center are on the destruction of human embryos (Volarevic et al., 2018). Another cell type, Mesenchymal stem cells (MSCs), are well-known in that can develop into

many different cell types as well as decrease immune response and inflammation in the body (Volarevic et al., 2018). However, because of these abilities, many are concerned with the capacity ‘to promote tumor growth and metastasis’ once put into a human or other organisms (Volarevic et al., 2018).

These and other ongoing debates and unfinished legislation laws are two reasons scientific research is trailing the evergrowing world (Marchant & Pope, 2009). For research to be conducted, proper legislation is needed. As of 2016, the FDA, which has regulatory control, has not approved any germline genetic therapy techniques (Grant, 2016). Stem cells are extensively utilized in research endeavors; however, inquiries arise regarding the appropriateness of certain cell line sources, prompting deliberations on their ethical utilization. Therefore, until there is substantial “freedom” to conduct scientific research and there is no established legislation, treatment options and promises from genetic editing technologies and stem cell therapies for VML are hypothetical.

**Methodology**

The approach to answer the proposed question to this research topic will contain information from primary and secondary sources. This falls under the Responsible Research and Innovation (RRI) framework for the methodology (Stilgoe et al., 2013). This framework addresses the societal duty of conducting scientific research to yield effective outcomes while prioritizing moral considerations (Stilgoe et al., 2013). This is closely intertwined with this research endeavor due to the obligation these domains bear in ensuring the development of dependable solutions within ethical parameters, as well as fulfilling the task aligned with the

initial intent. The sources mentioned throughout aim to understand how ethical concerns shape scientific research using genetic editing technologies and stem cell therapy. The sources used are first-hand experiments with primary data or reviews analyzing previous studies. Both will educate the reader on why these technologies are not used freely and will allow the reader to formulate their conclusions based on the research done.

**Literary Review**

Though stem cell therapy and gene editing techniques are emerging strategies for combating VML and other skeletal muscle ailments, ethical concerns still jeopardize the full potential of these options. The primary concern raised earlier revolves around the profound impact both approaches have the potential to exert on human lives. Numerous individuals contend that gene editing intrudes upon the natural order, assuming a role akin to that of a deity. Furthermore, embryonic origins remain the predominant source of stem cells. Resolving ethical concerns surrounding these practices proves challenging, as divergent perspectives on the utilization of human embryos are held with considerable gravity. For example, in their article discussing ethical concerns about stem cells, Lo and Parham explain how one population may say that the embryo is not a living being until placed in a woman's uterus, becomes a fetus, and then becomes a child. Others can argue that, at conception, the embryo has rights and a moral status (Lo and Parham, 2009).

Due to stem cells originating from embryos, ethical dilemmas drawback their potential. A large fraction of the embryonic stem cells (ESCs) are obtained from in-vitro fertilization (IVF) clinics where the embryos are demolished. These embryos are transported to a laboratory where

they undergo separation, incubation, and cultivation to expand their cell line (Landry and Zucker, 2004). Before these cells can be expanded and utilized, they must first be located. IVF clinics for studies and experiments use leftover embryos not used for infertility from donors – most do not know that their donation is going to science (Schaefer et al., 2012). ESCs are recognized as pluripotent, and able to differentiate into various cell types utilized in stem cell therapy studies, such as VML (Lo and Parham, 2009).

Based on studies using engineered tissue scaffolds seeded with cells – induced pluripotent stem cells (iPSCs), satellite cells, and forms of ESCs are the prime candidates for skeletal muscle regeneration (Shadrach and Wagers, 2011). ESCs are used in regeneration due to their amazing ability to reproduce and differentiate into many cell types (National Research Council (US) and Institute of Medicine (US) Committee on the Biological and Biomedical Applications of Stem Cell Research, 2002). One cell type that ESCs can differentiate into is muscle cells. Subsequently, these muscle cells can aid in the regeneration of skeletal muscle. (Akiyama et al., 2018). If these cells were used in scaffolds for future implantation, this could be a viable solution for VML.

Gene editing has been controversial since its beginning in the late 1900's. However, since the CRISPR/Cas9 technology was invented and used in labs, debates have heated up. CRISPR/Cas9 has been discovered as an easier, more user-friendly, and more reliable form of gene editing (*What Are the Ethical Concerns of Genome Editing?*, 2017). One increasing fear of CRISPR/Cas9 is the safety factor of 'off-target effects' which are edits made in the genome where the technology was not targeting (*What Are the Ethical Concerns of Genome Editing?*,

2017). CRISPR/Cas9 has also attempted to modify embryos for therapy targets. The effects of gene editing are not perfect, therefore fears of altering the human genome are slowing the progressive ability of CRISPR/Cas9 in many areas (Joseph et al., 2022). This technology has been created to help cure many genetic-related diseases in patients including sickle cell anemia, Alzheimer's, and muscular dystrophy (Mani, 2021). The effects of this technology are long-lasting, therefore any mistake in editing genes could be detrimental.

Given the growing demand for regenerative medicine, CRISPR/Cas9 technology aims to address this need. When dealing with regenerative medicine, CRISPR/Cas9 will often work with stem cells to reveal new avenues for solutions. In an example involving amyotrophic lateral sclerosis, which can cause muscle dystrophy, CRISPR/Cas9 was used to alter iPSCs to correct a gene mutation leading to this disease (Dilip Kumar et al., 2022). The advantages of using stem cells and CRISPR/Cas9 combined foster new directions in regenerative medicine. Many studies are ongoing to see if CRISPR/Cas9 technology combined with stem cells can open new solutions for diseases and ailments like muscle dystrophy and VML. Commonly, iPSCs or ESCs are used with this technology because of their strong differentiation abilities.

**Discussion and Results**

As seen, CRISPR/Cas9 and stem cell therapies can become important in the world of regenerative medicine. Because the need is so vast, the hope is that the technology's growing popularity will overlook ethical concerns about changing natural human lives and the need for many medical ailments like VML. However, new options are being discovered that steer away from using embryonic stem cells and gene editing human genomes in living organisms in an

attempt to solve the issue. For example, to move away from ESCs due to ethical concerns, scientists and researchers have derived induced pluripotent stem cells (iPSCs). These cells are, unlike ESCs, 'morally superior' because they do not require the disembodiment of embryos (Volarevic et al., 2018). These iPSCs can differentiate as well as ESCs and are less likely to cause an immune response when used in patients because they can be derived from the patient's cells. However, because of their differentiation ability, they can create tumor cells that can lead to cancer (Volarevic et al., 2018). As with all discoveries that aim to help or cure a medical condition, trials and tests must be done to ensure safety. When considering a treatment that uses embryos or genetic engineering, it is common to tread lightly due to ethical concerns that halt the study's progress. Hence, scientists and researchers are attempting to find other avenues that do not contain questionable materials or techniques.

There is also concern about funding for stem cell research. According to the Genetic Science Learning Center at the University of Utah, '...political parties debate about how to fund stem cell research. The federal government allocates billions of dollars each year to biomedical research. But should taxpayer dollars be used to fund embryo and stem cell research when some believe it to be unethical? Legislators have had the unique challenge of encouraging advances in science and medicine while preserving a respect for life' (*The Stem Cell Debate: Is It Over?,* n.d.). If there is little to no funding for the research, that can stop the advancements fast. Interestingly, funding can come from those who carry ethical concerns about these technologies. However, though many may disagree about funding research, most scientific studies receive government grants or private organizations where those concerned do not have much say. On that matter, legislation is the primal ruling on permitting such research.

In the light of genomic editing for medical conditions, most believe this can be beneficial when used in the right conditions and with respect. Legislation is highly recommended to make sure there are no cases of misuse but, most countries including the U.S. and Europe agree that this technique should be used if the "medical benefits are highlighted" and any effects to mom and baby are decreased to a minimum (Joseph et al., 2022). In an outline to review the concepts of genetic therapy and the ethical concerns that go with it, Eisenberg and Schenker state in the abstract that 'society must determine its attitude toward germline alteration and toward intervention for the purpose of genetic enhancement. Eugenic genetics is purely theoretical at present and is likely to remain so for a long time. Articles in the press, sometimes influenced by specific pressure groups, generate public fear that is in most cases unfounded, due to the lack of feasibility of performing the claims voiced in them' (Eisenberg & Schenker, 1997). When talking about stem cell research, a large concern when using ESCs is the possibility of human cloning and going 'too far' in the lab when dealing with these fragile cell lines. However, like genetic editing, if correct procedures are followed and protocols are enforced, the benefits can outweigh the fears of this technology. A paper discussing the ethical concerns surrounding stem cells pointed out a thought that is quite truthful, "... moving forward with the right blend of creativity and caution is essential, in the interest of both science and patients. In all areas of stem cell research and therapy, nuanced consideration and discussion of the best translational pathways, as viewed by ethics as well as science, will play a vital role in balancing hope and hype now and in the future" (King and Perrin, 2014). This notion holds credibility across various aspects of the medical field, particularly concerning the earlier topics. With careful progression in these fields, these choices can yield significant impact.

When utilizing the methodology framework, Responsible Research and Innovation (RRI), it is imperative to prioritize understanding and adhering to the concept of responsibility. Those seeking to employ these techniques must recognize the weight of the responsibility involved and acknowledge that these tools are not to be taken lightly; they possess the potential to significantly impact humanity if mishandled. Within this realm of responsibility, the outcomes derived from these experiments ought to be dependable and aligned with the highest interests of everyone, even outside of the experiment.

**Conclusion**

Ethical considerations have been demonstrated to impede the advancement of relevant scientific research crucial for enhancing current patient treatments. When contemplating individuals afflicted with various skeletal muscle disorders, including VML, and lacking adequate treatments for improved quality of life, ethical considerations hinder the overall advancement of genetic editing technologies and stem cell therapies. This hesitation may render these therapies seemingly obsolete due to concerns about potential harm, notwithstanding the benefits outweighing the drawbacks. Why fight the use of stem cells and genetic editing when the good outcomes that will come out of them will help so many patients? To ensure these technologies' safe and effective utilization nationwide, it is imperative to enact and enforce appropriate legislation, thereby preventing any ethical lapses. The initial step is often the most daunting, yet once their potential benefits for humanity become evident, the medical landscape will undergo a transformative shift.

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