Gene Drives in CRISPR Technology, Mosquitoes to Eradicate Malaria, and Implications in Jewish Medical Ethics

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"When man gives himself to the covenantal community, the Halakhah reminds him that he is also wanted and needed in another community, the cosmic-majestic, and when it comes across man while he is involved in the creative enterprise of the majestic community, it does not let him forget that he is a covenantal being who will never find self-fulfillment outside of the covenant and that God awaits his return to the covenantal community."

—Rabbi Joseph B. Soloveitchik

Abstract

With the discovery and advancement of CRISPR technology, gene drives have become more effective and precise, presenting new opportunities for disease prevention. CRISPR gene drives promote the inheritance of specific traits, significantly altering the genetic makeup of an entire population. CRISPR Gene drives have been proposed or used in case studies for practical purposes, with the most actively researched area involving controlling the spread of vector-borne infectious diseases.

Malaria, which is transmitted by vectors, is a significant public health concern. Malaria alone kills over 650,000 people each year while afflicting 200 million more with debilitating fever. Malaria is caused by a *Plasmodium* parasite and is transmitted by female *Anopheles* mosquitoes. With the use of CRISPR gene drives, there are aims to reduce the populations of malaria-transmitting mosquitoes through either a suppression drive or a modification drive. A suppression drive is used to reduce the number of malaria-transmitting mosquitoes by altering the insect's reproductive capabilities and a modification drive is used to make the insects resistant to certain diseases by changing genes in mosquitoes to prevent them from carrying the malaria parasite.

When investigating the potential applications of CRISPR gene drives in eradicating malaria through mosquitoes, it is imperative to address ethical concerns in both secular and Jewish contexts. Some of the concerns raised include unintended consequences, animal welfare, altering the natural order of creation, *kilayim* (prohibited crossbreeding), and *Pikkuach Nefesh* (the obligation to save a life). The evaluation of CRISPR gene drive applications should prioritize transparency, accountability, and public participation to effectively assess the potential advantages and disadvantages of this powerful technology.

PART ONE

Genetics

Deoxyribonucleic acid (DNA) is a sophisticated molecule that houses the genetic material required for all living things to function. Every cell in an organism's body has almost identical DNA. Although the majority of DNA is located in the cell nucleus (nuclear DNA), a small amount can also be discovered in the mitochondria (mtDNA). DNA consists of a long chain of nucleotides, which are composed of a sugar, a phosphate group, and one of four nitrogenous bases: adenine (A), thymine (T), cytosine (C), and guanine (G). The nucleotides are arranged in two long strands, forming a spiral that is commonly called a double helix. The structure of the double helix is compared to a ladder, with the base pairs serving as the rungs and the sugar and phosphate molecules acting as the vertical side rails. The genetic code, which is in charge of encoding the instructions required for the growth of an organism, is determined by the sequence of these nucleotides [1].

An organism's or cell's genes are segments of DNA that contain the instructions needed to make a specific protein or RNA molecule that serves a particular purpose in the cell or organism. Genes are the basic building blocks of heredity -- each gene has a specific position on a chromosome, known as a locus, where many alleles, or variations of a gene, might exist. These alleles can have different DNA sequences, which causes variations in the expression of features like eye color or susceptibility to disease. Organisms that reproduce sexually have two copies of each gene, because each parent cell or organism donates a single copy of its genes to its offspring [2]. The patterns of inheritance of these alleles can be described using principles such as Mendelian genetics, which describe how genes are passed down from parents to offspring.

Mendelian genetics, proposed by Gregor Mendel in the mid-19th century, describes how traits are passed down from parents to offspring and how their expression can be predicted. Genes are inherited in pairs from each parent, with some being dominant and others recessive. Using Punnett squares, the probability of a trait appearing in offspring can be determined based on the genotypes of the parents [3]. The ability to manipulate genes and potentially correct genetic disorders has been made possible by recent developments in genetic technologies, such as CRISPR gene editing.

The History of CRISPR

In the late 1980s, a team of Japanese scientists who were researching bacterial immune systems revealed the initial discovery of CRISPR. They were looking at the *iap* gene, responsible for alkaline phosphatase isozyme conversion in *E. coli*, and noticed unusual repeating patterns in the DNA sequences of some bacterial genomes, which were interspaced with short, non-repetitive sequences [4]. A group of scientists led by Ruud Jansen and Francisco Mojica proposed the name "CRISPR" in 2002 for the distinct family of clustered repeats that Ishino and colleagues had identified a decade earlier [5].

The repeating nucleotide sequences of CRISPR sequences are typically 20–50 base pairs long and are separated by spacer sequences, which are typically 30–50 base pairs long. The repeating sequences are palindromic, which means they read the same both forward and backward, and they frequently have short, distinctive sequences on either side that are referred to as "flanking repeats." Later research by numerous research teams revealed that bacteria and archaea with CRISPR systems were immune to viral infection, in which spacer sequences were discovered to be essential for this resistance.

Researchers hypothesized that the CRISPR system works by capturing and storing short fragments of viral DNA within the bacterial genome. These fragments are then recognized by the host and can target and destroy incoming viral DNA. It was discovered that the CRISPR system cooperates with Cas proteins, which function as molecular scissors that cut the viral DNA and stop it from replicating [6].

In addition to its natural function in bacterial and archaeal immune systems, the CRISPR-Cas system has been found to have enormous potential for use in genetic engineering and gene editing. The Cas9 enzyme recognizes and cuts specific DNA sequences, allowing researchers to target and modify specific genes with a high degree of precision. CRISPR has been developed to function in a variety of creatures, including mammalian cells, allowing researchers to make targeted modifications to the DNA of living organisms with unprecedented ease and accuracy [7].

The Development of CRISPR

The modification and editing of the eukaryotic genome is challenging due to its extreme complexity and billions of DNA bases. However, by utilizing components of the microbial immune system, the powerful technology of CRISPR has developed in recent years.

Clustered Regularly Interspaced Short Palindromic Repeats, or CRISPR, is a process that was initially discovered in the DNA of microbes to recognize and eliminate specific viruses. The CRISPR system uses an enzyme called Cas9, which behaves like a pair of molecular scissors and can remove a virus from a bacterial cell. Short guide RNAs (sgRNA), which have about 20 base pairs, are also used by the CRISPR system to point the Cas9 enzyme in the right direction for cutting. The guide RNA is made so that it will be specific to only that one sequence that is

targetted, improving the chances that the DNA will be cut at that site and nowhere else in the genome [8]. The Cas9 enzyme precisely cuts the DNA sequence where it is needed, allowing the potential change in the DNA sequence. By making it simple to accurately insert new sequences with the addition of a DNA template or disrupt targeted genes, CRISPR has transformed genetic engineering.

Nevertheless, CRISPR has numerous drawbacks despite its enormous potential. The efficiency of the editing process may not be 100% when cells incorporate CRISPR, which may restrict the technique's effectiveness. Off-target edits, when CRISPR unintentionally changes unwanted portions of the genome, are another danger. Even though they are uncommon, these off-target effects can have serious repercussions. Therefore, despite the fact that CRISPR technology has a lot of potential, these issues must be resolved before it can be completely utilized for therapeutic purposes. To make CRISPR a safer and more reliable tool for precise genetic engineering, more research and development are required to enhance delivery strategies, boost editing effectiveness, and lower the risk of off-target effects.

Beyond editing somatic cells, the majority of the body's cells, it is also possible to manipulate the genetic material of gametes, such as eggs and sperm, as well as early-stage embryos, known as germline editing. However, any modifications made would not only impact the individual but also their offspring. Additionally, there is concern that germline editing could be used to enhance desirable traits rather than just treating disease. The utilization of gene drives invovles gerlmline editing. As gene drives entail tampering with an organism's germline cells, any modifications made could be passed on to succeeding generations.

In 2015 a partial ban on genome editing in human germline cells was put forward by scientists, given the huge ethical and societal ramifications of this technique [9]. A report by the

National Academies of Sciences and Medicine in 2017 suggested that germline editing should only be used in extremely rare situations, with strict regulation and transparency [10]. Therefore, gene drives are often studied in insects, rodents, fish, plants, and mammals. The most notable of studies is to reduce the populations of malaria-transmitting mosquitoes in order to combat a disease that still infects 200 million people and half a million deaths each year [11].

The Functionality of CRISPR

The CRISPR system uses a single-guide RNA (sgRNA) to direct the Cas9 enzyme to a specific location in the genome. This sgRNA is designed to be complementary to the target DNA sequence. When it attaches to the DNA, it activates the Cas9 enzyme to cut the DNA at that location. This causes a double-stranded break in the DNA, which the cell's built-in DNA repair system can then fix [12].

Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are the two methods for repairing a double-stranded DNA break. Without using a homologous template, NHEJ simply rejoins the two broken ends of the DNA molecule. This procedure frequently causes minor insertions or deletions at the site of the break, which causes nucleotides to be added or removed at the location of the break, known as "indels." If these indels occur in the genome's coding regions, they may result in unintended alterations that may interfere with the operation of crucial proteins and enzymes [13]. Therefore, NHEJ is seen as a risky method of genome editing, yet it can be effective in disrupting genes.

HDR, on the other hand, is a more accurate repair method that bridges the gap of the double-stranded break using a template DNA molecule. This makes it possible to accurately modify the DNA sequence in order to add new genes or correct disease-causing mutations. The

most common form of HDR is homologous recombination. It is important to note this difference, in which HDR needs the existence of a template DNA molecule with homology to the target site, which makes it less effective than NHEJ [14].

Synthesizing a Recombinant Plasmid

Investigating the process of genetic recombination is important to understanding how a donor template can be incorporated into another genome. In bacterial cells, there is a single, double-stranded, circular chromosome alongside segments of circular DNA called plasmids [15]. Bacterial plasmids may encode genes for traits that are sometimes beneficial to their hosts, such as antimicrobial resistance, virulence, heavy metal tolerance, and the catabolism of unique nutrient sources [16]. With genetic engineering techniques, specific DNA segments from external sources can be added to a plasmid to produce recombinant plasmids [17]. Similarly, these DNA segments can be inserted into the bacterial chromosome, generating a recombinant genome.

Furthermore, restriction enzymes and DNA ligase are both necessary for this process to occur. In order to cut the DNA at a given location, restriction enzymes identify a specific site within the DNA and act as molecular scissors [18]. The cut can be blunt, straight down the middle, or with overhangs known as "sticky ends" [19]. Then, DNA ligase functions as a binding agent, "gluing" newly synthesized DNA segments together. When DNA sequences are cut and pasted using these two tools, the result is the creation of recombinant DNA molecules with the desired properties.

As a result, in order to produce a recombinant plasmid, the restriction enzyme first recognizes and then cuts the DNA fragment at a particular point --depending on the enzyme, this

cut may produce a blunt end or a "sticky end," which leaves overhangs of unpaired DNA. By using the same or a different restriction enzyme, the plasmid is also cut at a specified location, resulting in complementary ends that can join with the ends of the DNA fragment. The two pieces of DNA are then joined together using DNA ligase to produce a hybrid molecule that has the desired genetic information [20] [21].

In order to build a recombinant plasmid using CRISPR, a guide RNA that targets a particular gene sequence must be created. This guide RNA must be combined with the Cas9 enzyme that can cut the DNA at the specified spot. The target cells are then introduced to the recombinant plasmid, where it can be utilized to make specific alterations to the genome.

In the case of gene drive technology, the synthesis of a recombinant plasmid is an important step in creating the specific genetic elements that can spread a desired trait through a population. The desired gene can be incorporated into a plasmid, (which will replicate independently of the host genome) and then inserted into the germline cells of the target population. Of course, to create a gene drive system, scientists first need to identify the specific genetic sequence they want to introduce into the population. For example, this could be a gene that confers resistance to specific diseases, such as malaria in mosquitoes or to introduce genes that decrease the fertility of a population, which can aid in reducing the number of invasive species [22].

Gene Drives

The laws of Mendelian inheritance explain how genetic traits are passed down from one generation to the next. These principles state that each individual organism has two copies of each gene (or allele) and that only one copy of each gene is passed on to the offspring when the organism produces gametes. The allele that is passed on is selected randomly, with each allele

having an equal probability or a 50% chance of being passed on to an offspring, regardless of whether the allele is dominant or recessive [23].

A gene drive modifies genes so that those genes don't follow the typical rules of Mendelian inheritance. In normal inheritance, there is a 50% chance that any particular gene will be passed from parent to offspring. On the other hand, gene drive technology turns a 50% chance into a nearly 100% guarantee [24]. This is dubbed super-Mendelian (>50%) frequencies [25]. As illustrated in Figure 1, Gene drives significantly raise the probability that a specific set of genes will be passed down to the following generation, enabling the genes to spread quickly through a population and surpass natural selection.

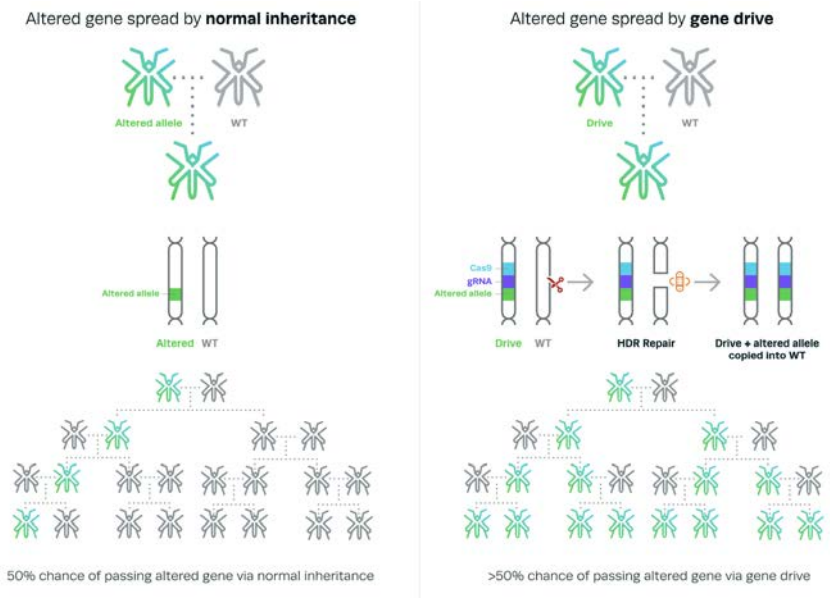

Figure 1. Normal inheritance (L) vs gene drives inheritance (R) [23]

There are two types of gene drives: natural and synthetic. Natural gene drives are found in nature and they can only develop through regular evolutionary processes. For example, transposable elements are mobile pieces of DNA that insert themselves into different parts of the genome and may inactivate genetic elements at those locations. However, synthetic gene drives are generated using various genome engineering methods, but they usually need to be

incorporated into the target organism during embryonic development before being further developed and released into the environment. The use of CRISPR technology has increased the potential for creating synthetic gene drives [23].

Gene Drives and CRISPR

CRISPR components can be incorporated into a "drive allele" that cuts a specific site on a homologous chromosome. Therefore the drive allele consists of three components: the desired gene to be propagated, Cas9, and the CRISPR guide RNA. The genetic material that encodes for these three elements gets inserted into a host DNA, in place of the naturally occurring gene one desires to replace in both chromosomes [24].

When an organism carrying the drive allele mates with an organism that does not, the offspring gets one copy of DNA from either parent – a gene drive version and a natural version of a gene (a gene is found in its natural, unchanged form is termed wild-type) [26]. When the sperm meets the egg and the chromosomes from the different parents line up for the first time, CRISPR in the gene drive DNA is activated. It recognizes the copy of the natural gene in the opposite chromosome, and the guide RNA directs the Cas9 enzyme to cut out the natural version of the gene before embryonic development begins.

Once the natural gene is damaged, the cell's repair mechanisms start working. With homology-directed repair (HDR), the repair machinery restores the missing DNA, but it uses the unbroken chromosome, which is the drive allele, as its template. So when the repair is finished, both chromosomes carry a copy of the gene drive. The drive allele is successfully copied into the wild-type chromosome, completely replacing the wild-type DNA sequence at this position in the genome.

From that point on, two identical copies of the gene drive, one on each chromosome, will be in every cell and the organism will pass the gene drive on to the next generation, so offspring will inherit the alteration and the gene drive. Future generations will carry out the same process. Every time the drive allele is passed on, CRISPR cuts the natural version of the gene, cell repair machinery intervenes and one copy of the gene drive becomes two, causing the alteration and the gene drive to spread through the population. The new gene spreads across the population in a matter of generations, occasionally completely replacing the naturally occurring gene [23] [24] [25].

It is important to note that when the target site is cleaved, instead of the break being repaired by homology-directed repair (HDR), it can be repaired by the non-copying competing non-homologous end-joining (NHEJ) pathway. NHEJ often results in imperfect copying, generating drive-resistant indel (insertion/deletion) alleles [27]. Therefore, a challenging requirement involves ensuring that the cut sequence is repaired using homology-directed repair (HDR) to copy the drive allele rather than (NHEJ) pathway. However, HDR rates are known to vary across cell types, developmental stages, species, and phase of the cell cycle [28].

Applications of CRISPR Gene Drives

Gene drives have the potential to be used primarily in three distinct fields: human health, agriculture, and the environment. Within these areas, the most actively researched area involves controlling the spread of vector-borne infectious diseases.

In the context of human health, vector-borne infectious diseases are diseases that are transmitted to humans or animals by vectors, such as mosquitoes, ticks, fleas, or other arthropods. These vectors can carry pathogens, such as viruses, bacteria, or parasites, and

transmit them to humans or animals through their bites. Malaria, dengue fever, and Lyme disease are a few examples of diseases spread by vectors.

Malaria alone kills over 650,000 people each year, most of them children, while afflicting 200 million more with debilitating fever [29]. Malaria is caused by a *Plasmodium* parasite and is transmitted by female *Anopheles* mosquitoes. *Anopheles stephensi* and *Anopheles gambiae* are two different species of mosquitoes that are known to transmit malaria. Vector-borne diseases such as malaria are a major public health concern, especially in tropical and subtropical regions where they are endemic [30]. However, as a result of changes brought about by international travel and climate change, mosquito-borne diseases are no longer confined to tropical and subtropical countries. Therefore, to eradicate these diseases, CRISPR gene drives are used to attack the mosquitos that transmit them.

The wild-type sequence on one chromosome is replaced with the drive allele, housing three components: the desired gene to be propagated (altered allele), Cas9, and the CRISPR guide RNA. There are two general types of drive alleles that can be designed: a modification drive or a suppressive drive. In suppression drives, a genetic alteration that reduces population size is spread. They are referred to as suppression drives as they function to prevent reproduction as opposed to directly causing animals to die. In a modification drive, the desired altered allele simply spreads by “hitchhiking” with the drive to wild-type chromosomes [23].

Therefore, to reduce the number of people infected with malaria, a suppression drive is used to reduce the number of malaria-transmitting mosquitoes by altering the insect's reproductive capabilities, or a modification drive is used to make the insects resistant to certain diseases by changing genes in mosquitoes to prevent them from carrying the malaria parasite.

In a suppression drive, an altered allele is spread that is undesirable to the organism (for example, causes infertility in females) when two copies are present (homozygous recessive). When the drive is copied in the germline, each offspring remains a heterozygote. As a heterozygote, they are minimally affected by the genetic alteration, enabling the genetic alteration to spread quickly through the population at first. However, once the alteration becomes more common, homozygous offspring will be produced and the population will collapse.

In a modification drive, the altered allele (for example, resistance) spreads to wild-type chromosomes by "hitchhiking" with the drive. If the offspring copy the drive allele during the early zygote stage, all of the offspring's cells will be homozygous for the altered gene. However, if the drive is only active in the offspring's germline all gametes will contain the drive allele, but cells that make up other tissues will still be heterozygous [23].

Case Study Suppression Approach: CRISPR Mosquito Gene Drives Eradicate Malaria

In this study, CRISPR gene drives were used to sterilize female *Anopheles gambiae* mosquito vectors by targeting their ability to reproduce. Using a sterility index, three candidate genes with high ovary expression and tissue specificity were chosen to act as probable female fertility genes in *Anopheles gambiae*. The three identified genes were AGAP005958, AGAP011377, and AGAP007280. The CRISPR gene drive would specifically target and spread the damaged female fertility gene, resulting in a recessive female-sterility phenotype, causing female mosquitoes carrying two copies of the altered gene to become sterile. Green Fluorescent Protein (GFP) was used as a marker to track the spread of the damaged genes and to easily identify them from wild-type mosquitoes.

The drive allele in this study was referred to as the CRISPR^{h} allele. The CRISPR^{h} allele, with the damaged female fertility gene and CRISPR components of Cas9 and gRNA encoded, was used to target the corresponding integration site on a wild-type (WT) chromosome. The CRISPR^{h} allele was resistant to nuclease cleavage, as its target sequence had been interrupted by the insertion of the CRISPR^{h} allele itself. During gamete formation, the target sequence was cleaved, initiating homologous recombination repair events that led to the spread of the CRISPR^{h} allele into the wild type allele.

The crossed heterozygous parents carrying the CRISPR^{h} allele with wild-type mosquitoes were analyzed for inheritance patterns of the CRISPR^{h} allele in mosquito populations. In the first generation, super-Mendelian inheritance with rates of 94.4–100% was found. Investigating further, the second generation too was also successful in converting wild-type alleles to CRISPR^{h} alleles, with rates ranging from 87.3% to 99.3% across the three target genes.

In those rare progeny that did not have a CRISPR^{h} allele, evidence of DNA repair by processes other than homologous recombination (a form of HDR), such as nonhomologous end joining (NHEJ) was examined at the three target loci. NHEJ often results in small insertions or deletions in the DNA sequence at the site of repair – a total of 13 indel mutations were found in a total of 32 offspring derived from a minimum of 7 individuals, presumably arising from NHEJ or other repair systems [13] [31] [32].

Case Study Modification Approach: CRISPR Mosquito Gene Drives Eradicate Malaria

In this study, CRISPR gene drives were used to pursue a population modification approach with the introduction of genes that confer a parasite-resistance phenotype to *Anopheles stephensi* mosquitoes.

In previous studies, the anti-malaria genes have been developed for the parasite Plasmodium falciparum: malaria vector mosquito *Anopheles stephensi* was genetically engineered to make two mouse antibodies that successfully blocked the mosquito's ability to transmit Plasmodium falciparum [33]. In this study, the same group paired the anti-malaria genes with the CRISPR gene drive, which enabled those genes to be edited automatically into a precise location in the genome of all of the mosquito's future offspring. The introgression of an effector gene at a high enough frequency in a vector population would decrease or eliminate transmission and result in measurable impacts on morbidity and mortality [34]. These mosquitos, therefore, are transgenic, which refers to an organism that has had its genetic material altered by the introduction of genes from another organism using techniques of genetic engineering.

The drive allele in this study was referred to as the pAsMCRkh2 construct. The pAsMCRkh2 construct contained the genes for the CRISPR gRNA and Cas9 enzyme, a gene for DsRed fluorescent protein (belonging to the family of GFPs), and two antipathogen effector genes (m2A10-m1C3). Additionally, there were also DNA fragments to facilitate the insertion of the gene drive into the genome of the mosquito. The relatively large size of the gene drive pAsMCRkh2 construct was approximately 21 kilobase pairs (kb) in length, with the specific components to be inserted into the *Anopheles stephensi* genome target locus comprising a total of 16,625 base pairs (bp) out of the 21 kb length.

Using a system that scanned the insects' eyes for the DsRed fluorescent protein, two males were identified DsRed+ for carrying the gene drive. When these two transgenic males were bred with wild-type females, the first male line spread their malaria-blocking genes producing all DsRed+ adult progeny, whereas the second male line produced 44% DsRed+ progeny. When bred again, extreme non-Mendelian DsRed segregation patterns were evident in

both male and female outcrosses. The first line produced 1,321 (99.7%) DsRed+ and 7 DsRed− larvae, whereas the second line produced 4,631 (99.2%) DsRed+ and 35 DsRed− larvae. There were efforts to reduce the activity of the nonhomologous end-joining (NHEJ) pathway to favor HDR-mediated insertion of the pAsMCRkh2 construct. However, through gene amplification and sequencing, the DsRed− larvae confirmed NHEJ pathway activity by evidence of target site-specific deletions.

The data presented here establish a CRISPR gene drive system that is target-specific, copying from one chromosome to the other with ≥98% efficiency while maintaining the transcriptional activity of the genes being introgressed. However, a potential downstream concern would be the development of resistance because the DNA construct works by forcing the mosquito to express a pair of antibodies against the malaria parasite, and changes in the parasite's genome might render it no longer susceptible to these antibodies [35] [36].

PART TWO

Ethical Considerations and Concerns Regarding Non-Human Applications in CRISPR and Gene Drives

George Lucas, an American Filmmaker, proposes the following question that can be applied in the context of CRISPR and gene drive technologies: “How do we handle uncertainty, including especially the uncertainty attached to novel developments, new technologies, and contrasting or competing ways of living—how do we grow comfortable with the new and the novel? How, in particular, do we discern the appropriate governing principles, or ‘rules of the game,’ when we don’t know precisely what the ‘game’ is, let alone its rules?” [37].

The highly cost-effective technique of CRISPR Gene Drives offers many potential advantages and applications in fields such as public health, wildlife preservation, and agriculture. As explored above, gene drives offer an important tool in the fight against malaria but also include other deadly diseases such as dengue fever and Lyme disease. Furthermore, this application has the potential to control or alter not only mosquitos, but a wide variety of animals, such as rodents and bats, invasive plant pests, and reservoirs [38]. As a result, this technology has unprecedented power that may save millions of lives each year. However, it's also crucial to take into account both anticipated and unanticipated risks [39].

The discussion around CRISPR gene drive serves as a reminder that major ethical implications extend to other living creatures we share our planet with. Regarding the potential environmental impacts of eradicating mosquitoes' ability to transmit malaria, on one hand, it is possible to anticipate future applause for the eradication of malaria. However, on the other hand, unless properly controlled and regulated, this research presents a concern because it might quickly change our ecosystems and organisms in negative and permanent ways [40]. Therefore,

because gene drive systems are designed to alter the environments we share in ways that will be hard to anticipate and impossible to completely revert from, questions such as about the ethics surrounding the use of this research are complex and will require very careful exploration [41] [42].

Since 2015, scientists have published four proofs-of-concept showing that CRISPR gene drives could spread a targeted gene through almost 100% of a population of yeast, fruit flies, or mosquitos, with most research to date focused on controlling (suppression drives) or altering (modification drives) mosquitoes that transmit malaria to humans [37]. Despite that, in 2017 the National Academy of Science, Engineering, and Medicine asserted that proof-of-concept in a few laboratory studies to date is insufficient to support a decision to release gene drive modified organisms into the environment due to permanent risks to ecosystems and organisms [43]. Since gene drives will eventually have an impact on every organism of the entire species once they are in place, scientists are developing and creating important safety "off-switch" features, such as unique ways to control, suppress, reverse, or eliminate gene drive systems from the population in the event of an unexpected or emergency event [44].

In order to better understand the ethical concerns of the study, it will be useful to backtrack and analyze the ethical considerations related specifically to CRISPR alone.
The concept of genetic engineering is frequently met with opposition from most people because it is “unnatural”, constitutes “playing God”, and is simply something “humans should not do” [45]. These claims do not all raise the same issue, but one widely accepted understanding of why genetic engineering is unnatural or constitutes playing God, is that it involves “crossing species boundaries”: the insertion of genetic material from one species into the genome of an individual from another species. Species boundaries are understood as naturally established, divinely

ordained, or morally and biologically significant, and genetic engineering disregards this important boundary [46].

CRISPR offers an alternative response to the objection. CRISPR does not necessarily involve the genetic material of another species. Instead, it utilizes a naturally occurring DNA repair pathway to alter genetic information. Therefore, the resultant animal is not transgenic. However, this is not the case with CRISPR gene drives, specifically using modification drives. In the modification approach, transgenic mosquitoes were created by introducing antipathogen effector genes, which were sourced from another organism, into the genetic material of the mosquito species *Anopheles stephensi*. Nevertheless, there is an important distinction between modification and enhancement that needs to be taken into consideration. Using CRISPR gene drives to eliminate diseases and for therapeutic purposes is different from modifying these technologies for enhancement purposes [37].

At the same time, no matter the intention or purpose, animal welfare–the state of the animal– is still in question. However, what constitutes as animal's welfare, and what methods should be used to determine how well an animal is doing? There are three commonly accepted major components of animal welfare: mental well-being, physical health, and engagement in species-typical behaviors that can provide insight into how an animal is doing. Additionally, it is common to treat death as harm to the animal, therefore a negative welfare impact [46].

The impact of the CRISPR genetic engineering technique on the welfare of animals was evaluated by Dr. Marcus Schultz-Bergin. The precision of CRISPR, in comparison to past techniques, is often identified as one of its key revolutionary features [47]. By using a guide RNA to help direct the Cas9 cutting enzyme to the correct location, significantly reduces the risk of off-target edits in comparison to conventional non-targeted genetic engineering techniques.

For that reason, Dr. Marcus Schultz-Bergin expresses that due to the increased precision of CRISPR, there is potential for improved animal welfare. With the minimization and possible elimination of off-target edits, fewer engineered animals will suffer from negative health and other effects resulting from gene editing. It is worth noting that not all off-target edits have detrimental effects, as there are instances where a gene sequence can be altered or removed without any observable changes in the phenotype [46].

It is a reality, nevertheless, that CRISPR can still result in off-target edits. A recent study that used whole genome sequencing to test for off-target mutations demonstrated the ambivalent nature of CRISPR's efficiency [48]. While CRISPR's high efficacy enables it to induce the desired mutation in a greater number of cells than previous techniques, it also increases the likelihood of an off-target edit if the Cas9 protein binds to the wrong location.

These off-target mutations result mainly because the guide RNA is not specific enough. The guide RNA is 20 nucleotides long, but can guide Cas9 to DNA sequences that differ by as many as five nucleotides from the intended target [49]. Furthermore, not only is it important to consider the frequency of off-target edits, but it’s critical to take into account what results from the off-target edits as well. As a result, even if CRISPR significantly reduces off-target edits compared to previous techniques, it might not be reassuring if these off-target edits result in significant harm [46].

Even more so in a CRISPR gene drive system, these off-target gene effects can potentially be passed to subsequent generations, harming future organisms and the environment. These uncontrollable genes, which weren't initially introduced by CRISPR but emerged as a result of its application, could lead to a significant rise in harmful mutations [37]. With CRISPR's risk of unintended genetic expression, this may have negative impacts on an animal’s welfare.

For example, suppression drives that genetically engineer mosquitoes to be sterile in order to eradicate the population mostly neglect the suffering of the animal, risking the acceptance of negative welfare effects in favor of other aims. However, modification drives may propose a more sensitive approach to animal welfare, by merely introducing disease resistance into wild animal populations [46].

As time progresses, CRISPR gene drives will continue to evolve and improve, leading to more advanced technology. This may reduce some risks, such as off-target effects, and increase the therapeutic potential of this system [37]. As previously stated, it may be possible to engineer technologies that will reverse or stop gene drive [44]. However, these advancements may introduce a new set of ethical concerns and consequences. For example, if gene drives are reversible and CRISPR has no off-target effects, is it still ethical to eradicate all human diseases in favor of a genetically homogenous population? Who decides whether someone can release a gene drive that can transgress national borders? Despite investigating an analysis of ethical considerations, there still remain numerous ethical concerns that give rise to conflicting questions.

Exploring the Intersection of Science and Judaism: An Interdisciplinary Approach

In order to properly address the ethical concerns and implications of gene editing from a Jewish standpoint, it is essential to first analyze the intersection of Judaism and science.

The Rambam, a prominent Jewish philosopher and physician in the Middle Ages, stated that “commensurate with the knowledge [of God] will be the love [of Him]” [50]. It is explained from this quote that the Rambam holds the two commandments to love God and to stand in awe of God are fulfilled through scientific inquiry and the accumulation of scientific knowledge.

Through scientific study, one learns to appreciate God's wisdom which results in love, and at the same time, understands the insignificance and lowliness of the human being in the grand scene of cosmic order, which results in awe [51]. Therefore, the analysis of the scientific world within the framework of the Torah is approached with the perspective that scientific study enhances religiosity. In addition, the Gaon of Vilna also emphasizes the importance of mastering science in order to understand the Torah "because Torah and science are bound up together" [52].

As mentioned before, the origins of gene editing technology originate from bacterial immune systems in regards to how bacteria protect themselves from being infected by pathogenic viruses. It was discovered that bacteria were able to recognize and delete foreign genetic viral DNA to inactivate the pathogens.

The fact that gene-editing technologies were discovered from studies in bacteria supports a broad interpretation of the Mishnah in Pirkei Avot that states "*Hafokh ba va-hafokh ba, de-kula ba*" which means "Delve into it and delve into it, for everything is in it" [53]. The traditional interpretation of this statement is that all of the wisdom of the world can be found in the Torah, leaving it to Torah scholars to diligently study and clarify the specifics. This concept can be applied as a Torah perspective on science since the study of how God structured the laws of nature can lead to the development of new human therapies. For example, the study of the biology and genetics of animals, plants, and microorganisms can produce knowledge that leads to the development of new medical interventions, such as gene editing [54]. In the context of tampering with DNA, the Talmud affirms that the answers to curing diseases lie within the laws of nature because God created the cures for all diseases even before He created disease pathology [55]. "*Hafokh ba va-hafokh ba, de-kula ba*."

Given the complexity of the science underlying gene editing, Rabbis who address issues pertaining to these technologies must have a solid understanding of molecular biology, molecular genetics, cell biology, and reproductive medicine. Before presenting Halachic perspectives, it is important to highlight that Halachic scholars have a long history of investing time in understanding science. For example, Mar Shemuel was one of many Talmudic scholars who had scientific or medical expertise. Additionally, in order to establish Halachic rulings, Talmudic scholars and poskim throughout the last two thousand years relied upon and consulted scientific experts. It is stated in Masechet Hullin 63b, that hunters and trappers were consulted by rabbis as reliable experts in identifying kosher birds. Furthermore, Sanhedrin (5b) states that Rav studied spent 18 months studying with a shepherd to determine which blemishes were permanent and which were temporary in order to establish the halakhic laws of *bekhor* (firstborn). More modern poskim, like Rabbi Shlomo Zalman Auerbach and Rabbi Moshe Feinstein, frequently consulted with Torah-dedicated medical scientists and doctors for technical guidance, enabling them to produce responses to concerns arising from advancements in science and medicine [54].

<u>Man's Role as an Active Partner: Permission to Intervene and Complete Creation</u>

Man's role is completion (*hashlamah*) of the process of Creation. Many midrashic sources indicate that man is an active partner with God in the process of creation and is hence responsible and entrusted with bringing creative processes to completion.

Beginning with the verse "תַּמְשִׁילֵהוּ בְּמַעֲשֵׂי יָדֶיךָ" (Tehillim 8:7), meaning "You have given him dominion over the works of Your hands," it is derived that although humans are also created by God, they are entrusted with the responsibility of managing and perfecting the world that God created [56]. God commanded Adam to “Be fertile and increase, fill the earth and master it; and

rule the fish of the sea, the birds of the sky, and all the living things that creep on earth." (Bereshit 1:28). Rabbi Dr. J. David Bleich comments that this verse seems to give Adam permission to engage in any form of conduct that is not specifically proscribed; God did not intend to prevent man from applying his creativity in finding new uses and purposes for the objects of creation and that there is no injustice to animal species or inanimate objects in doing so [57]. In the context of conquering disease, the Ramban and Rabbi Shimshon Hirsch understand this verse to mean that God gave man the right to use all natural resources, inanimate and animate, for use to improve the world and fight disease. Additionally, Rabbi Joseph Ber Soloveitchik expands this position by stating that all stories in the Torah point to a moral lesson for mankind. Bereshit's description of how God created the world acts as a moral command, instructing man to be creative and use his creative abilities to work alongside God to protect and better the planet [58].

Furthermore, Tiferet Yisrael (Rabbi Yisrael Lifshutz), states, "Any activity that we have no reason to prohibit is permitted in Halacha without having to find a reason for its permissibility, for the Torah does not mention every permissible thing but rather elaborates on only those things that are forbidden" [59]. That being said, Halacha does not need to spell out all permissible activities. Similarly, Rabbi Dr. Avraham Steinberg writes, "As long as the act of perfecting the world does not violate halakhic prohibitions, or lead to results which would be halachically prohibited, then we are given a mandate to use science and technology to improve the world" [60].

A midrashic story displays the philosophical principle that human beings are permitted, and sometimes commanded, to alter nature in order to perfect the works of God [54]. In this Gemara, the story took place in the second century C.E., reflecting a dialogue between Roman

tyrant, Turnus Rufus and Rabbi Akiva. The Roman tyrant posed three questions found in different parts of the Talmud. The first question in which the tyrant asked was 'If God wanted man circumcised, why did he not create him so?' Rabbi Akiva replied 'Because God created man with a symbolic imperfection in order that man should join with the Almighty in perfecting himself. In this way man becomes a partner with God in creation.'

The Roman tyrant continued, 'If God loves the poor, why does He not feed them?' Rabbi Akiva replied, 'Because God wants us to join with Him in perfecting society and so become a partner with Him in creation.' Finally, the tyrant's third question, 'Which are better, the works of God or the works of man?' Rabbi Akiva replied 'The works of man,' to which the Roman tyrant exclaimed 'Look at the stars in Heaven!' Rabbi Akiva confidently responded 'Look at the wheat, this is the work of God. Look at the bread. This is the work of man. Because real perfection is achieved when man joins his labor with God's work and in this way becomes a partner with God in creation [61].

Rabbi Akiva demonstrates to the Roman tyrant, through the comparison of kernels of wheat with man-made bread, that the works of man, as finishing touches to nature, are better than the unfinished works of God [54]. From the tyrant's perspective, the status quo was perfect. There is disease and death, as well as slaves and masters. Yet, every natural element is admirable, whereas anything unnatural is not. However, according to Rabbi Akiva, the natural only makes up half of creation because it is up to man to fulfill the remaining portion [61].

Rabbi Dr. J. David Bleich explains how God intentionally arrested the development of various creations before they could reach full completion. God could have allowed the wheat kernels to turn into flour and eventually a type of bread, however, God commands that humans must intervene. We must grind the wheat, combine it with water, knead it, and bake it in order to

make bread. Therefore, the process of creating bread is a joint effort between God's creation of the seed and human intervention to bring it to its final form [57]. God challenges man to collaborate with Him and finish His work not only in regards to bread, but in healing sickness, in caring for society, in exploiting the resources of nature, given that God guards, tends, and safeguards this world [61].

Although Jewish Law assigns and obligates man to use nature for the completion of the world, it also acknowledges that man can, through improper use of his free will, negatively interfere with the creation to the point of destroying both himself and the natural world. A midrash expresses, "When G-d created the first Man he took him and showed him all the trees of the Garden of Eden and said to him 'See my works, how beautiful and praiseworthy they are. And everything that I created, I created it for you. Be careful not to spoil or destroy my world — for if you do, there will be nobody after you to repair it.'" (Koheles Raba 7:13.) God set restrictions and limitations on man's behavior to keep him from harming or destroying creation [60].

This great partnership of "The heavens are the heavens of God" while "the earth has He given to the sons of man" (Tehillim 115:6) gives us the capabilities to develop the world of God. With this gift of dominion over the world and its inhabitants, man is given permission to use his intellect, creativity, and physical abilities to improve the world, as long as they follow the laws of the Torah with good judgment and acts of prudence.

"Playing God"

With our obligation to intervene in nature, which includes curing illnesses and improving the world, Rabbi Dr. Jason Weiner explains that the act of playing God can have positive

connotations, as long as the action is not prohibited, does not lead to prohibited behavior, and the benefits outweigh the risks. The Talmud states that after the first Sabbath, God granted Adam "creative knowledge similar to divine knowledge," in which he took two stones, struck them against each other, and created the first fire. These actions exemplify the idea that God has provided humanity with creative capabilities within nature, along with the wisdom to use them for the betterment of the world.

Rabbi Dr. Jason Weiner continues by stating "It is thus not being like God that is objectionable, but rather the 'playing around' with these God-like powers that are problematic. Talk of God in relation to these incredible scientific advancements should evoke reflections on wisdom, love, and altruism. When using the phrase 'playing God' regarding these incredible powers, the emphasis should be put on the word playing. Being Godlike within reason, in a responsible manner, is good. 'Playing' is not" [62].

The Jewish Emphasis on Saving a Life: *Pikkuach Nefesh*

Judaism grants humans the gift and authority to transform the natural world, but all of man's actions must bring the world closer to perfection and not further away. Although the impact of genetic engineering on humans and the environment remain largely uncertain, especially in the long run, the paramount value and extreme importance in Judaism placed on the preservation of human life may require to push beyond these limits [60].

The principle of saving a life, known as *Pikkuach Nefesh*, is rooted in the Talmudic teaching that "whoever saves a life, it is considered as if he saved an entire world." *Pikkuach Nefesh* is frequently referenced in discussions related to medical ethics and emergency situations.

The religious duty to heal is a priority that takes precedence over most biblical and Rabbinic commandments, emphasizing the importance of saving lives.

Genetic engineering has facilitated developments that have the potential to save and extend human life. According to Rabbi Nisson Shulman, this is a unique opportunity for humans to enhance and fulfill their responsibility towards God, this world, and the preservation of human life [61]. Additionally, in a conversation between Rabbi Dr. Jason Weiner and Rav Asher Weiss, Rav Asher Weiss advised that we shouldn't approach circumstances involving *Pikkuach Nefesh* with an excessive amount of caution. Rav Asher Weiss emphasized that when the goal is saving lives, we should work to encourage new technologies to advance as quickly as possible, but still with a lot of prudence and caution. He compared it to driving an ambulance in order to save a life. The ambulance should be driven as quickly as possible to reach the patient in need. However, it would be irresponsible to drive recklessly since that would jeopardize and reduce the possibility that the ambulance could save the person in need [62].

As we delve deeper into Jewish implications, both the suppression drive and modification drive do not *directly* save a Jewish life, nor the life of the mosquito for that matter. However, this technology holds the potential by a second degree to indirectly save lives, particularly in regions where adequate treatment for malaria is unavailable, through the use of CRISPR mosquito gene drives.

Genetic Engineering and CRISPR

One issue in regards to genetic engineering CRISPR technology is identifying the difference of medical and non-medical applications in CRISPR-based treatments.

The general consensus among Rabbis is that any potential Jewish ethical concerns about the technology will be superseded by the use of CRISPR for medical reasons, such as diagnosis and disease treatment. Many Rabbinical leaders in accordance with this view follow the well-known Mishna commentary of Tiferet Yisrael, which claims anything not explicitly prohibited in the Bible and Talmud is assumed to be permitted, as Halacha does not have to specify all authorized acts [58]. We cannot prohibit things simply based on the fact that they are new or strange [63].

The Jewish legal precedent permitting genetic manipulation for medical purposes arose recently with respect to mitochondrial replacement therapy [58]. According to Nishmas Avraham (4, p183), Rav Shlomo Zalman Auerbach stated that gene therapy is permitted and even praiseworthy for the treatment of hereditary diseases. As gene therapy aims to cure or prevent disease by repairing damaged genes, it is considered comparable to another healing procedure or medication and therefore permitted [64]. In this manner, the therapeutic application of gene editing to correct severe genetic diseases or life-threatening diseases would more likely be halachically permitted than would the use of gene editing to treat medical diseases where effective therapies already exist [54].

In the case of malaria, it is not a genetic disease and there is an effective cure with the use of prescription drugs. In addition, specifically in the suppression drive approach, the genes being replaced are not damaged– they are the natural genes of reproduction. Therefore, applying gene editing to mosquitoes to treat this genetic disease would be most likely not halachically acceptable based on the derivative of this precedent, as there already exist effective therapies and the question remains as to whether it can be considered therapy. However, the existence of drugs may not preclude CRISPR from being permitted, as there is no specific prohibition involved and

there is a possibility for treatment or cure to be effective with this technology. In the future, perhaps, if gene editing technologies are proven to be more effective than current medications, then Halacha may accept or allow gene editing interventions.

The commandment of *Pikkuach Nefesh* that everyone, especially physicians, must do whatever possible to save human lives, plays a role in ethically justifying the use of CRISPR in medical therapies and in diagnostic tests. According to Akiva Wolff, who is the research director of the Center for Judaism and the Environment at the Jerusalem College of Technology, comments that genetic engineering of animals and plants in order to save or prolong human life would certainly be permitted, if not required, by Halacha. An example Wolff provides is genetically modified organisms that can clean up spills of hazardous wastes. However, Wolff does consider how some expressions of genetic engineering may threaten and endanger human life and health. Those procedures that pose a significant risk to human life and health, either directly or indirectly due to their impact on the environment, may be prohibited by Halacha [60]. In regards to the discussion of mosquitoes eradicating malaria in order to combat a disease that still infects 200 million people and half a million deaths each year, although Judaism places great emphasis on the principle of *Pikkuach Nefesh*, since the ramifications on the environment and human health are still unknown, further research is necessary before a decision can be made [11].

Crossbreeding: The Prohibition of *Kilayim*

Another issue with the use of CRISPR technology for genetic engineering, specifically to animals, is the question of whether modifying a species with CRISPR should be considered a violation of the prohibition of *kilayim*, which pertains to the breeding of different species of animals. The focus of these laws is on the transfer of genetic material between different species,

not within the same species. Therefore, this specific issue will relate to the modification drive approach, which utilized transgenic mosquitoes.

The Torah prohibition of *kilayim* appears in the verse "You shall keep my statutes. You shall not let your animal mate with a different species; you shall not sow your field with different species of plants." (Vayikra 19:19) The prohibition of *kilayim*, according to almost all rabbinic authorities, the prohibition of *kilayim* applies only to Jews and not to non-Jews [60].

Rabbi Dr. J. David Bleich explains that Jewish tradition lacks a universal prohibition forbidding man from tampering with the known or presumed *teloi* goals of creation. Nevertheless, Rabbi Dr. J. David Bleich notes individual thinkers who have provided interpretations of specific commandments that imply such a prohibition. Some certain biblical commandments, such as those that prohibit crossbreeding of different species or the mixing of diverse agricultural species, could be understood to support this view.

Although Rashi considers the restrictions mentioned in *Vayikra* 19:19 to be *chukkim*, which are irrational laws not understood by human beings, Ramban disagrees with Rashi in his commentary on the same verse. Ramban believes that crossbreeding and prohibited mixing of species are prohibited because they constitute illicit tampering with creation. The Ramban states

> "Every creature and every plant is endowed by God with cosmically arranged distinctive features and qualities and is designed to reproduce itself as long as the universe endures. Interbreeding and cross-fertilization produce a reconfiguration of those distinctive qualities and also compromise reproductive potential. By engaging in such activities man usurps the divine prerogative in producing a new species or entity with its own novel set of attributes and, presumably, a species less than optimally suited to fulfill the divinely ordained telos associated with the original species."

According to the Ramban, as every creature has unique features and qualities that are cosmically arranged by God, crossbreeding would alter those distinctive qualities and compromise reproductive potential. Ibn Ezra's interpretation of the Torah's prohibition against crossbreeding is somewhat distinct. He explains that this practice is prohibited because it hinders the propagation of species, therefore unjust to the animals who are unable to carry out the divine goal of propagating their own species. On the other hand, similar to the views of the Ramban, Rav Hirsch viewed all *chukkim* as being indicative of the idea that humans should not interfere with the order, harmony, and purpose of creation [57].

While crossbreeding involves breeding two different species of animals, transgenic refers to an organism that has had its genetic material altered by the introduction of genes from another organism using techniques of genetic engineering. In the modification approach case study, the mosquitoes with the modification drive were transgenic, as antipathogen effector genes sourced from another organism were introduced to the mosquito's genome. The main distinction between transgenic and crossbreeding is the method utilized to introduce new genetic material. While crossbreeding involves selective *breeding* of different species, transgenic involves *inserting* foreign genes into the genome. Nevertheless, both transgenic and crossbreeding methods involve altering an organism's genetic material to achieve a desired outcome. Does the analysis of the prohibition of *kilayim* have any relevance to the case study involving transgenic mosquitoes?

It turns out, a very similar question was asked to Rav Shlomo Zalman Auerbach (Minchas Shlomo 2:100:7) whether the introduction of cell particles from one species into another, thereby changing its properties, constituted *kilayim*. There were differing opinions, with some suggesting that since cell particles are too small to be seen without magnification, they

might be exempt from the laws of the Torah which apply only to visible elements that can be seen by the naked eye (similar to the permissibility of consuming microscopic bugs).

Nonetheless, Rav Shlomo Zalman disagreed. He argued that the exemption for microscopic creatures does not apply to scientists who work directly and deliberately with these microscopic elements, as they are aware of their presence, and thus create the potential for *kilayim*. It is only the layman who cannot perceive microscopic creatures who may disregard them. However, despite this, Rav Shlomo Zalman did permit the technique in question. With respect to the prohibition of *kilayim*, the verse states "Behemtecha" (your animal), indicating that *kilayim* occurs only when two actual animals are crossed and not when microscopic elements of one are introduced into another [64].

According to Rabbi Dr. J. David Bleich, genetic manipulation that introduces a gene from one species into the genome of another species does not violate the prohibition against crossbreeding. Similarly, the Chazon Ish (Kila'im 2:6) states that the commandment is only violated if two living animals directly copulate. The Chazon Ish notes that artificial insemination designed to produce an interspecies offspring is not forbidden, just as transferring an organ from one species to another is not forbidden. Therefore, genetic manipulation, which does not involve a sexual act between members of different species, is not considered forbidden [57].

Furthermore, according to Akiva Wolff, most Rabbinic authorities rule that the prohibition of *kilayim* is restricted to the specific acts of crossbreeding spelled out in Vayikra 19:19. Therefore, the non-sexual transfer of genetic material between different species of animals or between animals and plants is not within the legal bounds of the prohibition [60]. According to these interpretations, modification drives in mosquitoes would not be prohibited.

However, this does not indicate that there are no ethical or moral considerations that must be taken into account. While the actual prohibition of *kilayim* is limited to Halachic definitions, the moral reasoning justification provided by the Ramban that we should not change the order of creation, must be respected. On the other hand, this does not imply that technology should not be used for the treatment and prevention of disease [63].

We do not need an explicit Torah prohibition to obligate us to think deeply about the moral implications of genome editing. We have an obligation to use our understanding to act correctly and morally. We have an ethical responsibility to consider the long-term effects that such actions could have on society, the environment, and ourselves. We must follow the general attitudes and values of the Torah, even without an explicit Torah obligation or prohibition.

Animal Welfare

Judaism has strict laws prohibiting any form of animal cruelty and preventing unnecessary suffering. Judaism opposes hunting and trapping for pleasure but allows animal use for commercial as well as therapeutic purposes, provided everything possible is done to eliminate or minimize the animals' pain. According to Jewish law, if you own an animal you cannot sit down to your own dinner unless you have first fed your livestock or pet [61]. One of the safety issues associated with CRISPR is the possibility of negative adverse effects if the wrong DNA site is targeted within a genome. Regarding CRISPR gene drive mosquitoes, in order to ensure adherence to the Jewish principle of kindness to animals, it is imperative to conduct careful experiments, ethical research, and maintain strict guidelines and oversight to minimize the off-target effects associated with CRISPR [62].

May the mosquitoes with CRISPR gene drives be patented or owned? In Judaism, the ownership of life forms is restricted. Specifically, human life cannot be owned, which prohibits both slavery and the sacrifice of one life for another. The donation of life-saving organs is only allowed after the moment of death. While Judaism does permit ownership of animals and other life forms, it places significant limitations and may even forbid the ownership of certain "types," such as a species. Therefore, the ownership of modified animals, such as mosquitoes with CRISPR gene drives, may not be permissible according to Jewish beliefs [61].

Conclusion

In order to move forward in a responsible manner, it is crucial that appropriate research is conducted, with an emphasis on establishing both safety and efficacy. All ethical concerns must be addressed, and it is important that that society as a whole, rather than just individuals or the scientific community, resolves and settles these serious concerns. Therefore, including religious communities in these discussions is especially important, as they can provide diverse perspectives and facilitate reflections related to meaning and values.

Wisdom is often found in the ability to recognize that just because we *can* do something does not mean that we *should* [62].In the second Midrash (Midrash Rabbah on Bereshit 1:1), it is questioned why the description of creation in the Torah begins with the letter "beit" in Bereishit bara Elokim, rather than with "aleph." According to *Chazal*, God saved the letter "aleph" for Halachic revelation in the first letter of the Aseret Hadibrot, *Anokhi Hashem Elokekha*. The moral message conveyed by this Midrash serves as a warning, reminding us that God's role in the world always takes precedence over humanity's desire for technological advancement and

discovery. Therefore, the success of technologies that we develop, particularly in the realm of CRISPR gene drives, is measured not by what we *can* do but by what we *may* do [54].

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