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Title: "Staging gene editing technology: An STS approach to the dominance of CRISPR"

Name of Student: Anastasia Xirofotou

Registration Number:13/219

Thesis Advisory Committee:

Aristotelis Tympas	Professor	HPS - NKUA
Katerina Vlantoni	Adjunct faculty	HPS - NKUA
Stavros Ioannidis	Adjunct faculty	HPS - NKUA

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“Πράγματι, όπως ακριβώς στην αστική κοινωνική πρακτική και στη συνείδηση που της αντιστοιχεί έχουμε αναγωγή της ποιότητας σε ποσότητα, του περιεχομένου στη μορφή, έτσι και η αστική φιλοσοφία και επιστήμη επιδίδονται από την γένεσή τους σε μια διαρκή προσπάθεια να συλλάβουν τον κόσμο με βάση ένα σύνολο από γενικούς νόμους που μπορούν να εφαρμοστούν επιτυχώς πάνω σε οποιοδήποτε τυχαίο αντικείμενο. Με άλλα λόγια, το τελευταίο θα πρέπει να μπορεί να «παραχθεί» από τους «μορφικούς όρους της δυνατότητάς του», από τους τυπικούς κανόνες νόησης..»

Κώστας Καβουλάκος από την εισαγωγή του βιβλίου
Η πραγμοποίηση και η συνείδηση του προλεταριάτου
Γκέοργκ Λουκατς- 1923

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Abstract

Gene editing technology is a relatively recent but rapidly expanding set of gene modification techniques. ZNF, TALEN and CRISPR comprise the main 'toolbox' of this technology. The discovery of CRISPR-Cas 9 (an endogenous bacterial defense mechanism) has expanded the gene editing techniques, rendering deletion, addition and modification of gene sites an easy and quick job. 'Crossing' horizontally almost every species (animals, plants, bacteria), gene editing and, especially, CRISPR is depicted as having 'revolutionized' the field of gene modification applications and capabilities. Since 2012 there have been very few relevant research fields that never dabbled in CRISPR. As of today, this technique is tested at the therapeutic level for the 'improvement' of animal species and the modification of plants. However, genetic modification is not a recent issue. Although the vision that stems from the intervention in an organism's genome –from recombinant DNA (rDNA) to CRISPR– seems to remain the same, by looking on which grounds one technique may be promoted over the other, the contingent scenarios that are enabled by different materials might become more visible. By centering the analysis on the way scientific comparison is conducted on papers and by filtering its conclusions through the STS lens, different assumptions and questions may arise regarding the terms of use, the performativity that accompanies them and the pertinent sociotechnical issues. This dissertation follows the means of comparison of the three basic gene editing techniques in two major scientific journals (*Nature* and *Cell*) in an attempt to understand what makes one technique 'better' than the other. It is also an attempt to understand the way science constructs its criteria, how these processes are articulated and how they can enable different realities that may lead to various ethical and technical issues.

Contents

1. Introduction

1.1. A brief history

Gene editing has emerged as a new scientific field over the last 15 to 20 years and consists of a set of techniques that permit targeted genome modification. Many papers now refer to its rather recent history as the 'gene editing era', underlining the major impact these techniques seem to have in all the applications that stem from gene modification. Stochastic interventions seemingly belong to the past and this appears to be the big difference between previous gene modification techniques (e.g. rDNA) and gene editing. Three basic techniques form the technical core of this new field: ZNF, TALEN and CRISPR. Although these molecules have a longer history, it took many other scientific incidents to come before them so that they can be transformed into techniques – not to mention a whole era. ZFNs (zinc finger nucleases) have been isolated in 1985 [1], [2], TALs were first described in 1989 [3], while CRISPR (clustered regularly interspaced palindromic repeats) have been detailed since 1993 [4]. Scientific endeavors and technologies like the human genome project, sequencing and recombination have transformed these molecules into techniques. The first important event that preceded and gave rise to gene editing occurred in 1994 when it was discovered that the double strand break on DNA, boosts the cell's intrinsic path of homologous recombination. A second important episode occurred when CRISPR was used as a gene modification tool in 2012 [5]. These three techniques have been deployed in this chronological order: ZNF, TALEN and, more recently, CRISPR.

In a different story line, gene modification has always been a topic debated by science, technology and society. As early as the first recombinant organism was constructed in a lab in 1973 through the use of rDNA [6], a historical attempt to define what risks, benefits, ethics and modes of regulation this new practice entails was conducted in the Asilomar congress [7][8]. Many years passed following this momentous event and the concerns around the safety and the possible uses of an engineered organism have faded away. It was not until the arrival of CRISPR that the scientific world made a comparable attempt to address societal concerns. In 2015, the International Summit on Gene Editing was convened by the US National Academies, the Royal Society in London and the Chinese Academy of Sciences in Washington, DC. Gene editing seems to have again brought forth all the relevant sociotechnical issues, yet in very different way. Ethical issues rather than risk and safety concerns are by now more prominent on the negotiation table. A significant attempt is made to meet present and future societal concerns and establish a public dialog around those issues [9]. Nevertheless, just before the 2nd International Summit on Human Gene Editing, on November 2018, a certain scientist announced that two human beings have been successfully edited [10]. Nana and Lulu were already

there through a scientist's words, touching the core of all the ethical issues at once. The first genetically engineered humans were a reality. In many ways, gene editing has not only produced novel perspectives regarding health, disease, ethics and governance, but also significantly accelerated existing ones, opening up great questions like the following: 'Now that scientists are able to do that, what should be made of it?'

"As a result, biomedical scientists now have a powerful remedy for a formerly crippling epistemological problem: in the absence of information, the human mind fills in the gaps with things it simply makes up. Genome editing is a tool to get away from these 'just so stories' and closer to biological reality. It is also the definitive tool for the **determination of causality**." [11]. Although the field of gene editing is nowadays mostly 'concerned' with the ethical and governance aspects, it is also presented as a means to fill the gaps of ignorance by achieving biological determinism. Based on this epistemological point of view, it is really important to have a look on the black box per se and see on which technical grounds this technology 'determines causality', what are the basic norms that characterize the field, what are the criteria upon which scientists make their decisions and how these materialities do realities.

1.2. Technical description of gene editing techniques based on the available literature

In order to understand more about these three techniques and approach the way that knowledge and progress is articulated on scientific papers, it is important to get familiar with how these techniques are presented (and are, therefore, thought) to work and how they are implemented. For this purpose, some pictures from the literature will be used but the analysis of how the different entities are represented goes beyond the scope of this thesis. Thus, they are only used to improve understanding and for familiarization with the scientific thought. Lastly, the basic terminology is explained in Appendix 2 (Glossary).

As a general principle, gene editing techniques are based on the so called 'fusion nucleases'. ZNF, TALEN and CRISPR are already-engineered molecules wherein a protein (ZNF and TALE) or an RNA (CRISPR) gets fused to an enzyme that has the ability to cut DNA (nuclease). More specifically, the big technical difference of those three is the DNA motif recognition part of the molecule. ZNF and TALEN are attached to the DNA through a protein that recognizes one to three nucleotides, while CRISPR attaches to the DNA through RNA, bringing together all the other components of the gene editing 'machine'. Although this seems to be only a miniscule part of how this technique is supposed

to bring the 'revolution', little attention is being paid to the intrinsic cellular mechanism of homologous recombination or the donor DNA. Deletion or addition of the right DNA is achieved through cellular mechanisms (homologous recombination and non-homologous end joining) that try to fix the DNA after being cut. It is through this repairing cellular procedure that a new piece of DNA which was put there by the designer, the donor's DNA, can be incorporated into the host's genome.

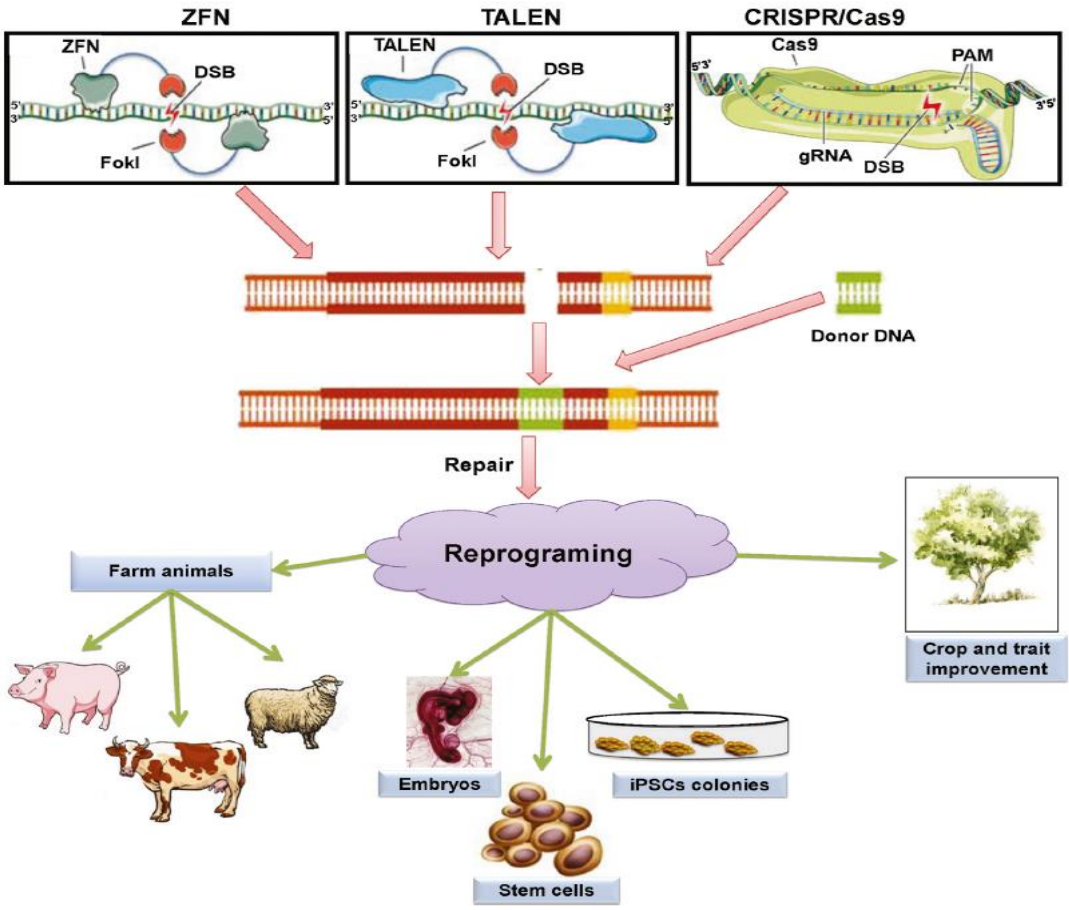

Figure 1: Schematic representation of how the three gene editing techniques work and their broad applications [12].

As presented in the picture above, all three molecules are provoking double strand breaks in the DNA and, through homologous recombination and the donor's DNA, the new right sequence is either incorporated or edited, leading to “better” animals, “better” plants and crops and, maybe, even to “better” humans. Although many questions arise from this picture (e.g. 'How come the DNA is just two straight lines? Wouldn't it be more accurate to put the cell at the center of the technique, since it is the most important part of the procedure? How is donor DNA found there and what is the meaning of the correct one?'), this lies beyond the dissertation's scope. The basic information to be extracted

is that gene editing is about a molecule that comprises of a motif recognizing part which leads the blind scissor in the targeted DNA sequence. The fact that RNA instead of protein is the leading motif recognition part in CRISPR seems to be its critical differentiation and buttresses its dominant role in the field.

1.3. The CRISPR era

One thing that is really striking about gene editing is the domination of the third technique, CRISPR. CRISPR-Cas 9 was used as a gene modification tool for the first time in 2012 [13] and, ever since, the terms “revolution” or “democratization” are associated with its name quite often [14],[15]. CRISPR has first been described as an immunity mechanism of bacteria, which tend to ‘save' some of the hostile viral genome sequences that attack them so that they can use these old viral remnants as a guide for the future. Thus, the next time the same biological entity attacks them, they are able to recognize it and cut it out.

Before 2012, ZNF and TALEN have also been used in several lab activities. Although these two molecules function differently, both are utilized extensively and have employed in several clinical trials [16]. With the initiation of gene editing, a tremendous increase in patent filings has been observed, but it seems to have taken off since the introduction of CRISPR. Each technique's potential to get commercialized has served as a very significant stimulus for the progress of the whole field. The 'modular' design of ZNF has permitted its rapid commercialization. Different biotech companies (Sangamo Bioscience, Sigma–Aldrich) have developed appropriate kits in order to automate the design procedure, delivering thousands of already engineered, ready-for-use proteins in the hands of scientists. Similarly, TALEN has also entered the market of custom-designed TALE arrays that were commercially available through Cellectis Bioresearch (Paris, France), Transposagen Biopharmaceuticals (Lexington, KY, USA) and Life Technologies (Grand Island, NY, USA) [17].

The political economy of gene editing is playing a crucial role concerning the terms of use and the overall progress of the technology. “In addition, commercially available ZFNs are dropping in price at the same time as methods developers are assembling tools to help researchers design their own nucleases. Meanwhile, TALENs and engineered meganucleases are also already commercially available. Which technology will dominate is not yet clear: there are still many unknowns, in particular about TALEN function as discussed in several of the pieces concerning this issue. But the price at which researchers in regular research labs can obtain good tools is likely to play a role” [18].

Shortly after 2011 and despite the relative easiness of design for ZNF and TALENS, CRISPR has been considered the dominant technique, as it made feasible every kind of gene modification. “In short, it's only slightly hyperbolic to say that if scientists can dream of a genetic manipulation, CRISPR can now make it happen. At one point during the human gene-editing summit, Charpentier described its capabilities as ‘mind-blowing.' It's the simple truth. For better or worse, we all now live in CRISPR's world.” [19]. It is more than obvious that the scientific world has fueled the expectations regarding this method. CRISPR is indeed used in many laboratories all over the world and several clinical trials (more than the previous two methods) have been launched. The number of patents has increased dramatically (figure 2) and the majority of them concern the commercialization of the technique by biotech companies so as to create reagents, cell lines and animal models [20]. In line with the substantial commercial interest, a Pubmed search of the keywords “genome-editing techniques” or “gene-editing techniques” in the last 10 and 5 years –unsurprisingly– yielded a total of 4,466 and 4,054 references with 890 publications for zinc-finger nucleases (ZFNs), 1,136 for transcription activator-like effector nucleases (TALENs) and 11,421 for CRISPR (n = 11,421) [21].

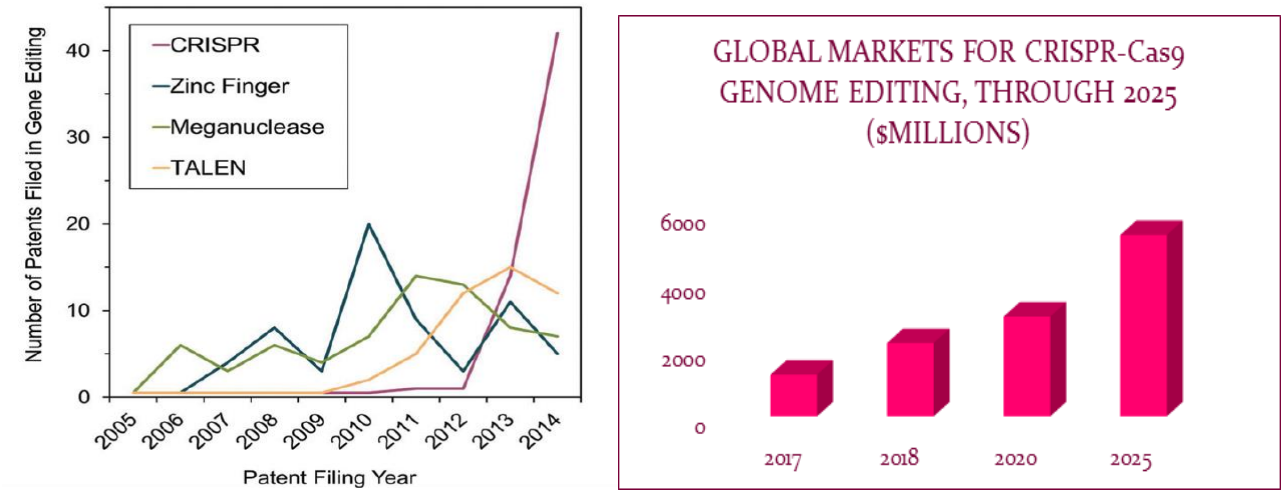

Figure 2: Number of patent filing based on the technique [9], expected profit for CRISPR until 2025[58]

Gene editing has been proposed as the most suitable term to describe the method in a study where ZNF were used to modify and produce a new human cell line, based on the analogy between a text and the DNA code [22]. Yeasts, bacteria and now human cells are in the race of lab programming equally. The 'simplistic' concept of finding a way to introduce a genetic tool into the cell (altering the transcriptionally meaningful parts of the DNA code and, eventually, altering the protein output into a functional one) has remained the same from the rDNA period. This concept is mostly dictated by the same vision of getting rid of the wrong DNA. No matter the species, the strong concept of genetic essentialism and biological determinism shows up either as therapy (when it comes to humans) or as

improvement (when it comes to plants and animals). What is at stake this time, though, is on what conditions the modification is taking place and how these techniques make this vision true, if they do so.

For the purposes of this dissertation, only the clinical part of gene editing applications (therapy) is taken into consideration. Taking a step back from all the ethical and regulatory issues, it is really important to understand better how CRISPR has become the most important tool; why this specific technique and not all the previous ones; what may the ideal circumstances of conduction be and who may be excluded by the affordances of this technique. As presented above, gene editing is depicted as a great opportunity not only for the scientific community but also for the industry and, perhaps, the medical community as well. Before taking the step of 'when' –that seems to be more relevant nowadays [23], [24]– it might be more important to redefine the risks and benefits of gene editing or have a look at how this concept may have been shaped, so as to bring back the questions of 'why'. Moreover, it might be helpful to have a closer look at the terms of comparison between the techniques and scrutinize how the succession is staged, in order to address this question in a meaningful way. All these important aspects can be raised only through the STS lens, which reveals the deep entanglement of the social and technical dimensions and shows how scientific progress is staged. By following a textual analysis that focuses on how arguments are staged and articulated in scientific papers, this thesis aspires to understand how gene editing has progressed and what are the main trade-offs between the three gene editing techniques.

2. Theoretical framework

2.1. Through the STS lens

The way that science –both as a practice and as an institution that explains nature and produces knowledge– is formed and functions in a historical context has been a very significant topic for STS [25]. T. Kuhn's 1962 work on “The structure of scientific revolutions” has played a tremendous role in the shaping of the STS field and has bequeathed us with a very contrarian account of what science does and how it does it. Kuhn has pointed out that science is a matter of the ‘paradigm' inside which it is borne and that only within this specific paradigm can its results explain and produce comprehensible knowledge. When many scientific results have no meaning or are better answered by an alternative theory, a scientific revolution takes place, rendering the previous system obsolete and void. Terms such as “incommensurability”, “paradigm” and “anomalies” have been a major contribution to STS and provide a very different view of science – or, better, of sciences. By following

science as a social-context-dependent practice of what scientist do and how they prove their comprehension of the world, a diverse network of people and institutions emerge. Scientific truth stands not as a universal value that should be served, revealed and safeguarded by scientists, but as a term that under different circumstances might not even be measurable. Another one of Kuhn's important contributions can be found in his “puzzle solving” mode of action. The sociotechnical and historical context in which a specific theory is framed not only provides scientists with the means of proof and measurement, but also generates the scientific questions in the first place. Just like a puzzle (which, at the same time, serves as the purpose and the means of solving it), a scientific method poses the question and, simultaneously, provides the means to answer it. If this opinion was to be taken into consideration regarding the ‘paradigm' in which modern biology is conceptualized, then what would be the questions and, therefore, the answers we are heading towards? Even though Kuhn's work has been criticized severely [26], it has nonetheless illuminated scientific progress in a very different way from determinism and opened it up as a historical process without ever questioning its truth.

There are several examples of technological and scientific changes that render progress a matter subject to social processes, during which it serves the ideological context where the different incidents take place. As Schatzberg has pointed out in his work about metal and wooden airplanes, the scientific community 'performed' the progress, as this was conceived at the specific historical moment [27]. What now seems as an inevitable and obvious result of an ever-progressive process, up until then was not that obvious. Metal airplanes, when compared to the wooden ones that were used in the 1920s, rendered equivocal results regarding the basic parameters of comparison: fire, safety, weight efficiency, cost and durability. Although the engineering parameters required a lot of effort, research and money to make metal (the “inevitable” succession from wood), this process was simply hidden from the rhetoric of progress. What Schatzberg showed is that progress is not an intrinsic characteristic of scientific changes or scientific community decisions, nor does it relate to the rational procedure of weighting the pros and cons. It was time to abandon the pre-industrial era and enter the era of precision, power and stiffness, where engineering would be more easily conceived as a scientific field rather than a craft. In this case, metal symbolized progress and served as the appropriate choice, even though the resulting practices would tell another story. Progress and power were bound to a specific material and, therefore, they were staged accordingly and not the other way around, leading to choices that are not very obvious or rational, as Schatzberg underlines.

Having that analysis in mind, several scientific practices and artifacts like papers, methods and scientific organs, take on a very different meaning. Following Kuhn's and Schatzberg's opinion, the

current perception of scientific facts should have happened at some point. What is today taken for granted (the assumptions, inclusions or exclusions of what is a fact, how it is generated and who comprehends it as fact) should follow the historical context and be socially dependent. Shapin's analysis of R. Boyle's experimental program, offers a very enlightening example of this procedure. On his article *Pump and Circumstance*, Shapin is stating that "In the setting of early Restoration England there was no one solution to the problem of knowledge which commanded universal assent. The technology of producing knowledge had to be built, exemplified and defended against attack. The categories of knowledge and their generation that seem to us self-evident and unproblematic were neither self-evident nor unproblematic in the 1660s. The foundations of knowledge were not matters merely for philosophers' reflections; they had to be constructed and the propriety of their foundational status had to be argued." [28] According to the article, Boyle, with his paper *New Experiments Physico-Mechanical* (1660), constructed a series of rules and conceptual terms that shaped both the modern criteria of scientific power and the form of communities that can be understood as scientific. These rules are: the production of results from a mechanical device, the repeatable testimony, the detailed description of the experimental conditions and devices and the distinction between de facto data (matter of fact) and probabilities to be investigated. Through Boyle's effort, the formal exclusion of any form of subjectivity from the experimental process was methodologically formed, and virtually any reference to its social character was concealed. Boyle's terms gave rise to what is nowadays perceived as universal consensus and, therefore, the basis for building a community with very specific characteristics; a community that has common assumptions (matter of fact) and can decide unanimously on the validity of certain possibilities, as it bases data's truth on this same criterion of universality and neutral representation. The use of passive voice and the probabilistic nature of the descriptions, the detailed descriptions of the experimental arrays, the use of reports to defend certain possibilities as data, the elimination of causal study in the realm of metaphysics and the controversies are the basic principles of modern scientific discourse and may not have been widely accepted at first, but nowadays count as state of the art.

What seems to be persistently excluded by this kind of data articulation (through technical texts of graphs, images and terms) is the laborious human process that yielded these results. If scientific papers are just claims about how nature is represented through specific machines that are used for specific mediations between the expert's eye and nature's laws, then who is generating the rules of what claims hold true? In addition, if this kind of information has purposely been excluded in the first place as subjective and irrelevant, then how irrational is Medawar's (1963) question whether "the scientific paper is a fraud?" [29]. As a biologist, Sir Peter Medawar questioned the established

narrative according to which data are articulated as things that already exist, concealing all the human effort that has been put into them and, on top of that, never letting these kinds of questions to show up. Like a self-driven process, facts are articulated as things that will eventually be included or excluded in the overall knowledge accumulation, by the realness they hold. In other words, essences and ontologies that are generated through scientific theories are objectified through some device and, at the end, they produce a specific representation or form. But someone might assume that different forms lead to different perceptions and it is neither a new methodology nor a socially independent process, the fact that science is 'squeezing' its motifs and knowledge through specific forms.

2.2. Research questions

This thesis tries to approach the techniques of gene editing from an STS perspective based on the previous analysis about science and its documentation. Although STS is not a unified study field with unanimous theories and aspirations (this would be demonstrably anti-STS), its methodology and a lot of its points about scientific progress will be put to use. As Schaltzberg has pointed out, sometimes scientific progress that is conceptualized as a technically proven argumentation carries a heavy ideological burden. Taking this point into consideration and in order to analyze why gene editing came to be such an important practice for scientists and, maybe, society, the first question that concerns this dissertation is:

1. How is gene editing's 'progress' articulated in scientific articles?

As previously presented, gene editing comes with a lot of noise, being a very significant scientific achievement and a big ethical question at the same time. In parallel to a discussion about the regulation and governance of how this matter can be handled by whom and for whom, it might be helpful to look on which technical grounds, these techniques have succeeded one another (if so). In order to achieve public engagement in the discourse around gene editing, some insights on the technical parameters that shape the field might be needed. If documents are artifacts that include references to the social processes through which they were produced and reproduced, then which are the social processes that shape the criteria in this case? In other words:

2. Which are the basic terms of comparison among the different techniques? Why (and how) has CRISPR emerged as the most important gene modifying toolbox, overshadowing the previous techniques?

By examining gene editing's articulation and how a technique may be better than the other, the

affordances of each one of them might become more visible and provide insights into its vision (and if there are different visions accompanying each one of them). If every method and artifact not only inform but also performs, what are these techniques doing and are there any differences between them? Putting it more specifically:

3. How gene editing shapes the field of its clinical applications and how is therapy conceived?

Through these questions, a deep sociotechnical entanglement between experts (technical) and non-experts (ethical) might show up and what is now presented as distinct areas of concern, might get interlaced through the lens of the contingent realities that this field may enable.

3. Methodology

The basic approach in order to understand how these three techniques that comprise gene editing's core are represented in the scientific literature and compared to each other will be textual analysis. This kind of qualitative analysis may reveal how gene editing's progress is represented and also which are the forms of representations. The analysis will take into consideration both narrative and represented data and will concern only the scientific papers that were included in the primary literature. The secondary literature will be used in order to capture the historical and sociological context of gene editing. In a nutshell, scientific documents and literature review in scientific journals were chosen as primary literature, gene editing's progress articulation and the terms of comparison between the three techniques were regarded as the main subject and textual analysis will be used as the basic methodology.

3.1. Secondary literature

The secondary literature that was used as a guidance for the implementation of this thesis was mostly based on papers and books that have contributed to or shaped the basic principles of STS. More specific opinions on the topic of gene modification and its historical aspects have also been used. A literature search on the topic of gene editing has yielded some very important results that helped me get familiar with the field's sociological history. The congress of Asilomar, an important historical landmark where society met scientists in an attempt to discuss the effects of rDNA, has been studied extensively. Treated as the only historical example that may “guide” current debate around gene editing to a fruitful discussion, it is not that surprising that it is still highly cited by social researchers and STS scholars [30], [31], [41]. In order to understand what was at stake in that congress (an event

that is still highly mentioned), I studied a variety of sources (such as the journal “Science for the people” [7]), papers that analyzed the synthesis of the congress and its results [30] and, also, landed upon the more recent points of view about the democratization of CRISPR, like Sheila Jasanoff's [31] and Maywa Montenegro de Wit's [32].

On a second level, I attempted to understand how gene modification is addressed by scientific institutions and how this issue is presented in non-technical scientific documents. Documents from institutions like the Department of Biotechnology, Ministry of Science & Technology of India [33], the U.S. Department of Health and Human Services, Centre for Medical Ethics, HELSAM [34], the Nuffield Council on Bioethics [35], the TA-SWISS Foundation for Technology Assessment [36] and the Max Planck Society [37], have been examined in an attempt to understand what are the issues that concern the scientific community regarding the use of gene editing.

Finally, under the general terms of research, more technical papers like opinion papers from EMBO reports [38], Nature Methods [39], the Science booklet on CRISPR [40], Cell Forum, Humanities and Social Sciences Communications – Nature [41] and Nature Biotechnology [42] have been collected and examined more thoroughly. All of the aforementioned secondary literature helped me understand the historical aspects of gene editing and the important events through which it has evolved, what is discussed and presented in the scientific community, what is discussed between STS and the scientific community and what matters are at the center of scientific concern. Through this rather diverse literature search, I managed to 'visualize' gene editing outside its technical scope and map the area in terms that would help the placement of this dissertation. In other words, the results of the secondary literature inspection helped me answer the question of “what are we talking about?” In very general and oversimplifying terms, the majority of the retrieved documents centered around the ethical issue of gene editing use on gametes and germ line modification, regulatory issues regarding the function of the technique on the clinical level and governance issues that pertain to the appropriate procedure and network that would be in position to decide (and would be considered the appropriate form of society's expression). Many important results came up and helped me pose the main research question of this thesis.

Another secondary literature source that has helped in the thesis's subject and its analysis comes from the teaching material that we reviewed during this year's courses and concerns the way STS sees science. It has been a really hard task to combine what is already 'answered' –or, better, questioned– from the STS point of view and the overall concern around gene editing, in order to address questions

that would be meaningful and could bring up connections between the technical and ethical facets of the subject. The most important secondary literature articles have been referenced in the first two chapters. That literature –along with my tutors' help and guidance– led me to the specific subject of the comparison between gene editing techniques.

3.2. Primary literature

Due to the initial intention of focusing on the way gene editing progresses and the technical grounds that this progress is measured on, the only journals chosen are publishing exclusively on biology topics. Although a lot of papers from sociology and the humanities have been used as a secondary literature, any non-technical document was excluded as extraneous to this thesis's scope. Additionally, given an attempt to get an the widest and most representative picture possible of what is discussed on the field, two of the most important journals have been chosen as the basic resources of scientific papers: Nature and Cell. Nowadays, a tremendous number of articles are published with respect to gene editing. A single search in the PubMed database using the keyword "gene editing" yields 15,227 articles. Most of them have been published after 2014. A similar research under the term "CRISPR" yields 19,083 results, with the time-frame statistical analysis showing an augmentation from 2014 onward. Following a spirit of enthusiasm, even journals dedicated to gene editing have been formed (e.g. the CRISPR journal). Among them, it is more than sure that different opinions must have been expressed, but based on the thesis spirit of following what scientists are saying and showing, Nature and Cell have been chosen as the most appropriate sources. The fact that Nature and Cell are publishing houses that include many journals and, also, the fact that a lot of them are top-rated (based on the impact factor list), are considered as an important characteristic that may permit a broader search of what is considered to be the state of the art for the biological scientific community and, therefore, the leading opinions that shape the field.

Another important factor that shaped the methodology was the intention to capture the way each of the techniques and their combination has been presented in the scientific literature. In order to have a closer look at their succession, a relatively big time-frame that would follow gene editing from the beginning was chosen. Therefore, the time-frame of search has been specified based on the use of the first technique of gene editing, ZNF. In 2005, ZNF has been used in human cells in order to correct an X-linked severe combined immune deficiency (SCID) mutation [48]. That publication has been marked as an important one, due to the introduction of gene editing as a potential therapeutic field for humans. It is important to clarify that many of the 2020 results haven't been included, as the search

was conducted on February 2020. Therefore the search's time-frame covers the last 15 years (2005-2019).

The keywords that were used for this literature inspection were “ZNF”, “TALEN” and “CRISPR”. The search was based on the assumption that the combination of those three keywords would be more appropriate in order to bring up the papers that compare them. The possibility that this search has excluded the articles which compare ZNF and TALEN (before CRISPR) was also taken into consideration. Therefore, three kind of searches have been attempted: the first one only with the key term “Zinc finger nucleases”, the second with “Zinc finger nucleases +TALEN” and the third one with “Zinc finger nucleases +TALEN+CRISPR”. In both databases, the advanced search online option has been used, in order to place the time-frame, the term(s) and the type of articles. As expected, a large number of papers has been retrieved and there also was a big overlap in the searches results. In order to narrow down the results, only review articles were included. Review articles are usually dense articles that narrate many data and tend to have more opinion-like conclusions. The first stage of selection was based on the paper's title and the year of publication. The majority was excluded by the title criterion, as many of them were referring to specific applications, difficulties or functions that each one of them has. It was also important to include articles that predated CRISPR, although not many were found that were focused on the comparison. The final step of inclusion was a quick review in the article's abstract. Articles focusing on comparison and their clinical applications were chosen. Fifteen articles fulfilled all criteria and were chosen as the primary literature for this thesis.

In the analysis that follows, the most important parts of these articles will be quoted and analyzed.. Some parts will be also highlighted with the use of bold letters. All of the terms and forms of comparison will be represented as they appear (e.g. textual analysis and tables) and the articles will be analyzed based on the year of publication, in order to make any trade-offs visible, track how progress has been staged and determine what are the groundbreaking features of CRISPR.

4. Presentation of primary literature analysis

4.1 Literature results focusing on ZNF (2005-2010)

In a 2005 article titled “Gene targeting using zinc finger Nucleases” [43], the use of ZNF and its brief history is presented regarding the way the molecule works and the potential advances over previous techniques of gene targeting. In a discussion over gene modification techniques some research studies on animal and plant organisms are presented and it is stated that “These studies indicate that ZFNs

will be powerful tools for making directed modifications in experimental organisms for functional studies and for creating models of human genetic diseases" [43, p969]. Previous gene targeting methods (mostly with viruses) are mentioned and despite the fact that many of them are characterized as important ones, there is a specific reference to the unfortunate event of leukemia development in two patients with SCID. Yet, the article introduces ZNF and its function to the potential territory of proper alternative for therapy hope. "This highlights the need for procedural adjustments, but the clinical success is very encouraging" [43, p970], is the interconnection statement between previous gene therapy techniques and ZNF. In a relevant reference of the in vitro experiment with ZNF and human cell lines, a 20% of success is mentioned, while the issues of toxicity and off-target effects are mentioned as potential restrains that need to be taken into consideration. Off-target effects are defined as unintentional DNA breaks that occur different sites from the targeted genetic area and are correlated with many dangerous and unpredictable events such as cytotoxicity and abnormal cell proliferation. Off-target effects are considered to be the reason of the observed cytotoxicity, which is a very general term to describe how toxic an action might be to the treated cells. The point that "it would be undesirable to create new mutations while correcting an existing one" and the fact that "it is known that DSBs (double strand breaks) are a source of oncogenic translocations" [43, p971], are posing the basic undesired effects that may result from the use of ZNF. The paper closes with the hope that the results presented can justify the optimism for the use of ZNF as an experimental tool that may help in biotechnology research and that "with further development, the ZFN strategy may be applied in the treatment of human genetic diseases". Delivery methods and a better understanding of the homologous recombination processes in human totally differentiated (somatic) cells are also mentioned as future "challenges", in order to "generate long term therapeutic benefits".

In a 2008 paper (*Zinc-finger Nucleases: The Next Generation Emerges*) [44], the "advances" and "challenges" of ZNF are discussed, while the article introduces its subject by stating that "Methods of modifying the human genome precisely and efficiently hold great promise for revolutionizing the gene therapy arena." [44 p1200]. The use of the terms "advances" and, most importantly, "challenges", reveals how possible drawbacks and limits of the technique is presented and represented by the authors. It seems that ZNF poses specific difficulties, which, through hard work, may be solved as they provoke the scientists to do so. As a benefit, it is mentioned that ZNF can stimulate gene targeting by several orders of magnitude and that its potential power is proved by the relatively high (29%) conversion frequencies achieved. Donor DNA is also mentioned as an important part of the technique's success that determines the type of correction that is to be made. As previously mentioned, the donor DNA is supposed to provide the correct template that is going to be incorporated in the

region where endonuclease will have provoked the double strand breaks. Although many things are mentioned about how the technique and the donor DNA are supposed to work, at the end of the relevant paragraph it is mentioned that the basic mechanism through which donor DNA is known to be incorporated (homologous recombination – HR) is a highly cell-type specific mechanism, while the presented experiments refer only to in vitro data. As in the previous article, the genetic locus of CCR5 is mentioned, this time under the term of "safe harbor" locus, where a very high conversion efficiency has been achieved in an in vitro cell line. The article continues to analyze the way that ZNF works through its existing models of "engineering platforms for ZNF domains". The ZNF domains refer to the subunits that are supposed to target the DNA and, therefore, are the key instructors of specificity. Then comes the analysis of two engineering modes, the "modular assembly" and the "context-sensitive selection strategies". ZNF is characterized from the fact that it takes two ZNF subunits to make the molecule work; it works as a dimer. The two designing platforms seem to render different efficiency results and this is a parameter that is taken into consideration. As in the first article, toxicity is mentioned as a drawback and as the discussion evolves and passes to the potential clinical applications of ZNF, the following limits of ZNF exploitation are mentioned: "(i) high DNA-binding specificity of the ZF domain; (ii) regulated
cleavage by the ZFN; (iii) efficient delivery; (iv) transient ZFN expression; (v) comprehensive evaluation of treated cells for potential ZFN-induced side effects; and (vi) assessment of the
potential immune reactivity" [44 p1202]. The article concludes that "The main advantage of using ZFN-stimulated gene targeting as compared to conventional gene-addition-type gene therapy **is the potential to preserve temporal and tissue-specific gene expression**" [44 p1205], and also closes with a reference to the clinical fail of previous gene therapy and the development of leukemia in the two patients with SCID, in an attempt to highlight the need for safer ZNFs before these enter the clinic.

In a 2010 paper where ZNFs are still on the focus, transgenic technologies are discussed. The title states the article's main subject: "Zinc finger nuclease technology heralds a new era in mammalian transgenesis" [45]. Transgenesis is another definition that still refers to gene modification, but highlights the introduction of a foreign genetic material into a host sequence. In this paper, ZNF is also compared to previous transgenesis techniques. The glossary provides a very interesting definition, which differentiates the traditional genetic approach and the approach of molecular genetics. ZNF and gene targeting in general, editing and transgenesis are approaches that are based on the norm of **reverse genetics**. The strategy of attributing a specific phenotype to a specific gene has changed into attributing a randomly found gene that has been initially identified solely by its position on the

genome sequence to a specific protein or phenotype. ZNF and the previous gene modifying techniques have been based on this strategy and they have made it work. Reverse genetics are constituted through these modification techniques that pinpoint a genetic locus by ‘messing' with it, in order to see what would go wrong (or right). Reverse genetics place gene and not protein or phenotype at the beginning of the causal initiation. A strategy resembling a ‘switch on and off' game is closer to this kind of genetics and ZNF is spearheading its staging. Moreover, ZNF is considered a “powerful technology that induces subtle mutations” [45 p134]. Several experiments on fish and rat animal models are mentioned and ZNF shows higher efficiency than the previous techniques. Once more, the need for a common engineering platform is also stated. The OPEN approach is mentioned as a common effort in different academic groups that has yielded “rapid, highly effective and publicly available method for engineering zinc finger arrays” [45 p137]. Off-target effects are also mentioned, but this time, data from ZNF experiments are also referenced. In contrast to the previous papers where off-target was only brought up as an expected side effect and possible clinical risk that should be taken into consideration, this article states that “no disruption of any of the 20 predicted ZFN off-target sites was observed” [45 p137]. Even though in the following text it is clearly stated that a more detailed understanding of off-target events is required, the word “predicted” delineates how these events are identified. Off-target activity of ZNF is calculated by “looking” on potentially risky genetic regions and ascertaining if they have been broken. A huge amount of assumptions and prepositions are accompanying this kind of analysis, which would require another thesis to be fully scrutinized. RNAi and lentivirus techniques are presented as very important and successful techniques but, overall, ZNF is deemed to be a more “attractive, faster, and perhaps less challenging alternative” that could be “applicable to any mammalian species and even to ruminants” and “has taken a major leap forward as a simple technology for gene knock-out (and potentially for gene knock-ins) and is now a reality” [45 p139]. Although this paper does not meddle with the clinical applications of all those techniques (which is quite obvious from the use of the term transgenesis), it concludes with the very honest expectation that the similarity of the concepts of gene therapy and transgenesis will lead to further technological “improvements”.

In another 2010 paper, the applications of ZNF solely are being reviewed [46]. In the abstract, reverse genetics (which have many applications in different animal models and human cell lines) are attributed to the ZNF application, which is regarded as, by now, ready to display its therapeutic potential. As a molecule that “**must** exhibit an extraordinary combination of qualities” [46 p636], ZNF is treated as a two-part tool that offers great versatility because it allows for the engineering of the two parts (the recognition head and the blind scissor) separately – a characteristic that simplifies

and enhances the designing and molecule engineering process. The issue of a common and efficient engineering platform tops this paper's narrative as well. Mechanism-based laboratory applications of ZNF are then described in detail. An analytical review of different animal and plant experiments compose the successful and efficient implementation of gene editing. Specificity with a subsequent risk of off-target events are also considered in this paper but, this time, only informational reference to the measuring methods that already exist and are under development has taken a place. The issue of how to measure these undesired effects is still ambiguous and bioinformatics seem to play a major role in the "prediction" strategy. As the authors bridge their argument about ZNFs application from bench to bedside, it is mentioned that two clinical studies have been initiated with ZNFs: one for the treatment of glioblastoma and the second for HIV. The so-called natural resistance to the HIV virus that occurs through the mutation of the CCR5 gene serves as the latter's trial goal. "[T]he potential advantage of a ZFN approach is a fully penetrant and heritable gene knockout (and consequent HIV resistance) that persists for the lifetime of the cell." [46 p644]. **Persistent, heritable, penetrant and lifetime** are three very specific terms that accompany the use and justification of ZNF use in the clinic so as to present it as a better option. At the same time, off-target events are still under discussion regarding how they are supposed to be measured. Finally, the paper concludes that "ZFN-mediated genome editing now offers the ability to carry out sophisticated gene-function studies **directly in the model system of interest**. [...] Thus, the application of current-generation ZFNs removes many of the constraints on experimental design that previously rendered some studies impossible and forced others to an achievable, but less than optimal surrogate. The addition of ZFN technology to our toolbox **will perhaps allow the awesome power of genetics to be extended to any eukaryote**" [46 p645]. Therefore, it seems that ZNF has allowed the application of gene editing in the most important "model" of interest, the human eukaryotic cell.

4.2. Literature results focusing on ZNF and TALEN (2012)

In a 2012 paper, TALENS (Tal Effector Nucleases) are placed in the frame of comparison. After 15 years of ZNF being in the spotlight, TALENS now emerges as an alternative solution [47]. A brief presentation of ZNF's functions and mechanism characteristics serve as the main introduction to the argument that although ZNF is great, there are certain limitations. "Clearly ZFNs are a very powerful resource for gene editing; however, there are some complicating issues with the design and application of ZFNs." [47 p1]. The main issues that are stated as ZNF's limitations are cytotoxicity and difficulty in efficient engineering of the molecule. What seems to be at stake is that efficiency is considered as a direct consequence of a good "design" and it seems that with the existing engineering

platforms “unexpected failure rates” have been observed. Platforms such as OPEN, which has been mentioned in previous articles, render the processes “arduous and time-consuming” and, over all, it seems that “some loci of interest may not be modified efficiently” with what was previously characterized as a “versatile” and “powerful” tool. In these grounds, TALENS emerge as a new technique that has “generated economically, sequence-specific DNA-binding proteins with predicted binding specificities in a matter of days, using molecular biology methods practiced by most laboratories” [47 p3]. Similar experiments in animal models are quoted with similar or higher efficiency compared to the ones mentioned about ZNF and may reach a 45% of transfected cells. It is also mentioned that TALENS are expected to provide greater flexibility over ZNFs, which makes this characteristic a desired feature. In conclusion, it is stated that after 10 years of “accumulated experience” over ZNFs use for gene editing, TALENS are the new favored option for efficient gene editing, despite the fact that several aspects need to be better concerning the TALENS mechanism of function: “(i) develop efficient means of delivery (TALENs are typically 1,200+ amino acids in length), (ii) define immunogenicity of TALENs, and perhaps most important, (iii) characterize the specificity of TALENs” [47 p3].

4.3. Literature results focusing on ZNF and TALEN and CRISPR (2013- 2019)

More recently, a 2013 paper displays the full spectrum of gene editing techniques. This article is mostly dedicated to ZNFs and TALENs. There, both of them are presented and each one's pros and cons are explained by the way they function [48]. “This combination of simplicity and flexibility has catapulted zinc-finger nucleases (ZFNs) and transcription activator like effector nucleases (TALENs) to the forefront of genetic engineering.” [48 p398]. A brief introduction to how ZNF binds to DNA and, therefore, the extent of programmability is again the main information around the technique, although, this time, beyond the two most popular engineering platforms (modular assembly and OPEN), the commercially available reagents and kits are also briefly mentioned. Proceeding with TALENs, the same narrative is still holding. TALEN DNA binding specificity is analyzed and presented with respect to its engineering potential. It indeed seems that TALENs are easier to construct, due to the fact that each TALE “head” recognizes not three but one nucleotide at a time. This “design” feature seems to “encourage ambitious endeavors”. Besides this advantage, other technical challenges were met, as stated elsewhere, and different engineering strategies (Golden Gate) were used to overcome them. Exploring the successful application of gene modification, it is stated that “these approaches support the study of gene function and the modeling of disease states by altering genes to mimic both known and as yet uncharacterized genotypes.” [48 p400]. As in previous

publications, a direct reference to the norm of reverse genetics is attributed to gene editing. Specificity and delivery methods are mentioned as issues that need to be improved upon. The way that these molecules are going to be delivered in a cell is treated as a major obstacle, as it has been correlated with mutagenesis, toxicity and low efficiency. Experiments on embryonic stem cells and induced pluripotent cell lines have been a very common substrate for editing experiments. Similarly, embryo microinjection for the generation of transgenic animal models is also placed in this array of progress. All of the above are mostly 'looking' at the lab life aspect of gene editing. With regards to the therapeutic vision, it is mentioned that "site-specific nucleases for therapeutic purposes represents a **paradigm shift in gene therapy**" [48 p401]. Unlike previous techniques that have reached the clinic, "ZFNs and TALENs are capable of **correcting the underlying cause of the disease**, therefore permanently eliminating the symptoms with precise genome modifications" [48 p401]. A full range of ZNFs direct applications in various diseases are then numbered. Last but not least, CRISPR shows up as the new added technique that merits further evaluation. CRISPR is described as a method that is easier to apply, as it is directly portable to human cells and has showed the capability of multiplexing. What is interesting in this paper's layout is the fact that the different features are presented in side boxes which are not intergrated to the main text. In Box 1, it is stated that the whole mechanism that these molecules are provoking in order to achieve the modification (the cellular mechanism of homologous and non-homologous end joining) is limited to particular cell types. Although this limitation is not very disturbing for research purposes, it makes a huge sense when it comes to the human body. Instead, the article makes a different proposal about the exploitation of some other cellular mechanisms which may be more universal and, therefore, more appropriate. In the second box, the delivery method problem is analyzed and it is stated that "Although site-specific nucleases provide a means for introducing diverse custom alterations at specific genomic locations, this technology is still limited by methods for delivering these enzymes into relevant cell types" [48 p402].

In a 2014 article, a slightly different analysis is presented [49]. As "knowledge of nuclease-specific features, as well as of their pros and cons, is essential for researchers to choose the most appropriate tool for a range of applications" [49 p321], this article serves as a guide for choosing the most appropriate tool. At the beginning, the common features of these three techniques are presented. By common features, the article presents the basic mechanisms that these molecules employ and the possible genomic alterations that they may lead to. In the second part of the article, an analysis of the three techniques is structured through three basic axons: composition, availability, targetable sites. In this context, ZNFs are characterized as "challenging to construct" and with "poor targeting density".

TALENs are “challenging and time-consuming”, while they offer a big advantage of targeting almost any given DNA sequence. CRISPR is characterized as providing a “simple design and preparation”. At the overall question of choosing the best programmable nuclease, the following table briefly describes the main determinants.

	ZFNs	TALENs	RGENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	*FokI*	*FokI*	Cas9
Success rate[‡]	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate[§]	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb×2	~3 kb×2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)

Figure 3: Comparison of three classes of programmable nucleases (original article's table quotation) [49]

In the table, besides the informational parts of techniques (e.g. DNA targeting specificity determinant and nuclease) CRISPR and TALEN are presented as the most successful, while they seem to have the highest mutation rate. Regarding off-target effects and cytotoxicity, ZNF is presented as the least safe one and described as having the higher off-target effects and cytotoxicity. In the basic text, the parameters presented in the table are condensed to the axons of specificity, multiplex, mutation signature (the pattern of mutations each one of them provokes) and delivery. At the end of the article, it is concluded that “Programmable nucleases have the potential to change the genetic landscape of life forms around us, including crops, flowers, fish, poultry, livestock, pets and humans” [49 p331].

In a 2015 article where the progress towards gene editing therapies is discussed, the techniques are categorized based on their functional DNA recognition into protein nucleases (ZNF and TALENs) and RNA nucleases (CRISPR) [50]. In a very brief comparison between the techniques, it is stated that “Cas9 protein is invariant and can be easily re-targeted to new DNA sequences and […] Another potential advantage of Cas9 is its ability to introduce multiple DSBs in the same cell (also referred to as multiplexing) via expression of distinct guide RNAs” [50 p122] giving a certain

preference to the CRISPR. Without taking too much space for the comparison of techniques' on the main text, the following table summarizes the pros and cons that each technique presents (figure 4): Specificity, targeting constrains, ease of engineering, delivery and multiplexing along with immunogenicity (as the only safety indicator) are the basic determinants where CRISPR seems to "win" – although the state of "unknown" is also mentioned in the section of immunogenicity. The word "ease" appears in four out of seven parameters that are presented as major determinants for comparison purposes, rendering how easy -therefore applicable- any of these actions may be (multiplex, design, engineer, deliver etc.) as a rule of thumb. As with almost every paper that was previously analyzed, the part of clinical application appears in the second part of the article. However, this time, factors that influence gene editing's therapeutic efficacy are also analyzed. A very specific characteristic that seems to be desirable is the "fitness of the edited cells". As the bedside part of gene editing is now in trial, human cells and physiology arise as a locus of challenges and desired features. One of them is fitness. In order for the therapy to be successful, the edited cells should acquire a feature of better adaptation and life prolongation over the unedited others. Another important feature is the ability to divide. The majority of adult and therefore fully differentiated cells are either in non or in really slow dividing phase, limiting gene therapy's application to only a few numbers of still mitotic adult cells. This article also presents the 2 main delivery methods to the human body: in vivo and ex vivo. In the first one, the editing tool is directly delivered to the target cells via injection, while, in the second, cells treated like 'Enlightened troopers' are initially isolated from the patients' body, corrected and then re-introduced to the patients' body to restore the diseased phenotype. Based on the previous analysis, this paper also narrates the basic successful examples of clinical applications.

	Zinc finger nuclease	TALEN	Cas9	Meganuclease
Recognition site	Typically 9–18 bp per ZFN monomer, 18–36 bp per ZFN pair	Typically 14–20 bp per TALEN monomer, 28–40 bp per TALEN pair	22 bp (20-bp guide sequence + 2-bp protospacer adjacent motif (PAM) for *Streptococcus pyogenes* Cas9); up to 44 bp for double nicking	Between 14 and 40 bp
Specificity	Small number of positional mismatches tolerated	Small number of positional mismatches tolerated	Positional and multiple consecutive mismatches tolerated	Small number of positional mismatches tolerated
Targeting constraints	Difficult to target non-G-rich sequences	5′ targeted base must be a T for each TALEN monomer	Targeted sequence must precede a PAM	Targeting novel sequences often results in low efficiency
Ease of engineering	Difficult; may require substantial protein engineering	Moderate; requires complex molecular cloning methods	Easily re-targeted using standard cloning procedures and oligo synthesis	Difficult; may require substantial protein engineering
Immunogenicity	Likely low, as zinc fingers are based on human protein scaffold; FokI is derived from bacteria and may be immunogenic	Unknown; protein derived from *Xanthamonas* sp.	Unknown; protein derived from various bacterial species	Unknown; meganucleases may be derived from many organisms, including eukaryotes
Ease of *ex vivo* delivery	Relatively easy through methods such as electroporation and viral transduction	Relatively easy through methods such as electroporation and viral transduction	Relatively easy through methods such as electroporation and viral transduction	Relatively easy through methods such as electroporation and viral transduction
Ease of *in vivo* delivery	Relatively easy as small size of ZFN expression cassettes allows use in a variety of viral vectors	Difficult due to the large size of each TALEN and repetitive nature of DNA encoding TALENs, leading to unwanted recombination events when packaged into lentiviral vectors	Moderate: the commonly used Cas9 from *S. pyogenes* is large and may impose packaging problems for viral vectors such as AAV, but smaller orthologs exist	Relatively easy as small size of meganucleases allows use in a variety of viral vectors
Ease of multiplexing	Low	Low	High	Low

Figure 4: Comparison of different programmable nuclease platforms (original article's table quotation) [50]

In this context, it is argued that certain diseases may be better addressed by gene editing therapy. As a general rule, diseases that affect the blood system (such as SCID and sickle cell anemia) are mostly ‘preferred'. This is mainly because they have been more studied and the main obstacle that was mentioned earlier, the delivery method, is better understood and overcome. More interestingly, it is stated that: “diseases in which edited cells possess a fitness advantage, so that a small number of engrafted, edited cells can expand and treat disease. One such disease is HIV, as HIV infection results in a fitness disadvantage to CD4+ T cells” [50 p127], providing sufficient explanation of why HIV is the most ‘tested' disease in the gene editing therapy.

In a 2016 paper, the most important clinical applications of all the techniques are presented [51]. In the first lines of the paper, the main vision behind gene editing is delineated through the following lines: “The realization of the genetic basis of hereditary disease led to the early concept of gene therapy in which “exogenous good” DNA may be used to replace the defective DNA in those who suffer from genetic defects” [51 p430]. It is also interesting that the term “new paradigm” is used in this article too: “the recent advent of genome-editing technologies has enabled a new paradigm in which the sequence of the human genome can be precisely manipulated to achieve a therapeutic effect.” [51 p430] As with almost every article, the first part is referring to the way these techniques work, with most of the focus reserved for the different genetic mechanisms they may propagate (gene disruption, deletion, correction, insertion) and the alternative engineering platforms that are focusing on the differences of DNA recognition and, therefore, binding of each one of them. Regarding ZNFs, although they are more “labor intensive”, they may be safer since this method takes into consideration the “context-dependent interactions” between DNA and the pattern recognition parts of the molecule. TALENs are easier engineered molecules that were quickly recruited to the field of gene therapy and are considered to be able to target almost any sequence in the genome. In the same context, CRISPR is also presented as a “highly attractive” method that is easily engineered and may act on almost any target in the sequence. Specificity, a major performance determinant, is thought to be higher for ZNF and TALENs, as these methods require dimerization. It is also stated it is still difficult to determine the full spectrum of off-target events and that most of the referenced studies are limited by the in-silico predetermination method. In the last part, a list of all the diseases that gene editing has been tried on as a potential correction therapy are presented and, although the number of clinical trials that were under implementation seem to

have grown, the majority of the research results are referring to in vitro generated data from human cell lines.

In another 2016 paper, where methods for measuring off-target effects are mostly on the focus, genome editing tools are presented as having revolutionized the exploration of new, emergent phenotypes and the introduction of new functionalities [52]. Enthusiasm pervades this article and expressions like “exciting tools to edit genes of interest with unprecedented **control and accuracy** in eukaryotic cells, paving the way for next-generation biotechnology” [52 p1] run through the text. Especially for CRISPR, it is mentioned that it has “revolutionized genome engineering applications due to ease with which they can be adopted to target specific gene” [52 p1]. The existing bioinformatic tools for assessing specificity and, therefore, identifying off-target effects are then analytically presented. As with many other publications, the paper's means of comparison between the techniques is condensed to the following table (Figure 5).

	Methods		
	ZFNs	TALENs	CRISPR/Cas9
Off-target activity	Low to moderate	Low	Low to moderate
Ease of application to generate targeted genome editing	Laborious, difficult, and substantial cloning protein engineering required	Laborious, moderately difficult, and substantial cloning required	Easy, simple cloning steps required
Ease of multiplexing	Low	Low	High
Ease of generating large-scale libraries	Low; laborious and complex protein engineering required	Moderate; laborious and substantial cloning required	Easy; simple oligo synthesis and cloning required

Figure 5: Comparison of the limitations of the genome editing tools (original article's table quotation)[52]

In this table of comparison, CRIPSR emerges as a clear ‘winner', having the least amount of limitations. Except from the first parameter that refers to the main subject of the article, the safety issue of off-target activity (where TALENs are presented as the less risky method), the other three parameters are categorized unded the terms “ease” of application, multiplexing, and generation of large scale libraries (where CRISPR emerges as the easier, less laborious and with high multiplex potential). In the text that frames the table, it is stated that ZNFs are thought to have the higher specificity due to the motif recognition pattern, but they often are cytotoxic, expensive and laborious. Accordingly, TALENs can target almost any sequence in the genome but are time-consuming, laborious to be delivered to the cell and have similar off-target effects. CRISPR is scalable and

affordable, but it also induces a lot of off-target events.

In a perspective 2016 article, the author claims that RNA-guided nucleases (e.g CRISPR) have democratized the field of gene editing. Unlike the two previous techniques, "CRISPR–Cas9 can be customized by replacing guided RNAs, making the system much more affordable and scalable." [53 p1573] Giving a brief story line of the emergence of gene editing through its techniques, ZNF is presented as having many "successful demonstrations" but high cytotoxicity, a problem which was later answered by TALENS. The latter have been catapulted "to the forefront of genome editing" but their dominance was meant to be short-lived due to CRISPR's rise. Following that narrative, CRISPR is presented as "eliminating the need for elaborate protein engineering or assembly" [53 p1574]and as having become the most popular tool, replacing the earlier techniques. On these grounds, and despite the fact that ZNF and TALEN are presented as having higher specificity and lower off-target events, the author describes all the possible ways to improve CRISPR's specificity and safety. The author concludes with the following: "It seems **inevitable** that, in the future, the genomes of many living things, including humans, livestock and crops, will be modified to enhance desired traits and to avoid unwanted phenotypes such as disease susceptibility, pushing us **toward a brave new world**. Whereas CRISPR–Cas9 is likely to remain the tool of choice in academia, both ZFNs and TALENs will find roles in future applications and innovations: to date, both ZFNs and TALENs have been successfully used in human clinical trials. Indeed, programmable nucleases, may eventually enable humans—products of evolution—to become **masters of evolution**". [53 p1576]. In this rather strongly expressed opinion, the vision behind gene editing seems to be diverging from that of a cure for bad genes.

In a 2017 article where the recent progress of gene editing is discussed, a more precautionary perspective is quoted [54]. Delivery, activity, specificity and the establishment of the manufacturing processes of the gene-edited cells are presented as hurdles that need to be addressed. The different genetic possibilities that were presented in previous articles are also described here, but this time they appear more as diverse therapeutic strategies. By separating the article in subparagraphs that deal with "choosing the correct DNA-repair pathway, the appropriate delivery vehicle, the suitable gene editing platform" [54 p415], the authors aspire to provide a guide for the application of gene editing in clinical practice. When it comes to the best gene editing tool, the inclination towards a tool that does not cause undesired mutagenesis and off-targets and would not have a negative impact on the fitness of the edited cells (like proliferation ad differentiation) is presented as an ideal situation. Specificity is s major determinant in this paper as well and, unfortunately, "[t]he low specificity of

the CRISPR–Cas9 system seemed to be a major predicament for therapeutic translation when the platform was initially used to edit the human genome" [54 p418]. Targeting range is also a factor that is examined in order to choose the right editing tool, but a relatively different opinion in comparison with previous articles is reached: "**The reason for choosing a particular platform for a particular application was, in most cases, probably based on the accessibility of a certain platform more than on their different biochemical characteristics**" [54 p419]. In contrast with the previous articles (where the biochemical characteristics of each technique has been analyzed in order to attribute different appropriate uses), in this text this is something that does not seem important. One interesting part of this article is the fact that it presents a small number of results from ongoing clinical trials. In a ZNF trial as an HIV treatment, even though it was successful and well tolerated, its overall efficiency was too low to produce long-lasting effects. Although several clinical trials are mentioned in the literature and the majority addresses clinical applications, very little references can be found. The risk benefit ratio is mentioned when it comes to the appropriate strategy planning, which is mostly conceived as the 'the worst the case, the riskier the treatment protocol': "But **if conventional therapies are already available**, such as treatment of HIV infection, a gene-editing approach in hematopoietic stem cells in combination with a cytotoxic-conditioning regime must persuasively demonstrate both the safety of the manufactured stem cell product and its potential to achieve a level of benefit that exceeds that provided by current treatment options" [54 p421]. In these sentences the author clearly states the inevitability of genome editing in therapy, even if alternative kinds of therapy are already available.

A more recent publications (2018), assesses gene therapy's clinical applications and difficulties [55]. As per usual, when the history of gene editing is recounted, it is mentioned that it was Cas9's speed, simplicity and low cost of the guide RNA design and engineering (along with its high activity and specificity) that established CRISPR-Cas 9 as a popular choice. In the part of the experiments analysis that preceded clinical applications, it is mentioned that toxicity issues have been corelated with the delivery methods and that, although the first experiments we easier, this was because they were applied to "easy" cell lines. The fact that the "platform" along with the donor DNA need to be delivered at the same time to one cell poses many toxicity issues. Another important issue for the bedside part of gene editing is that the cells that are extracted from the human body and are engineered and need to remain viable and proliferative (as long as they already are) for a long term. In their concluding remarks, the authors state that "**[t]here are many potential theoretical problems in moving genome editing into patients, but it is only by testing them in the clinic that we will learn about the actual problems, expected or unexpected, and begin working on solving them**. For

example, while there is much discussion of the potential off-target activity of the Cas9–gRNA platform, it remains unknown whether those off-target effects have any meaningful clinical relevance, and despite lots of discussion the definitive answer will come only through translation. Nonetheless, there are many reasons to be optimistic that soon an entirely new class of genetic therapeutics based on genome editing will be available to a wide range of patients." [55 p10] What is indirectly implied by the majority of the previous articles here is being stated clearly: gene editing can be staged as a therapy only through clinical experimentation or, put more bluntly, through human experimentation.

In a 2019 review article, the main subject is the comparison between the different tools. ZNF and TALENs are characterized as suffering from low efficiency due to their off-target effects [21]. On the other hand, CRISPR-Cas9 provides better efficiency, feasibility and multi-role clinical application. "The platforms for these technologies are improving every day, with a plethora of new data appearing due **to technology miniaturization and automation** and newer discoveries to improve the yield and specificity of an edited product" [21 p326]. The intense work on the field that was presented in many other papers is now rephrased with a slightly different purpose: automation. In the paragraph of the comparative analysis, the main results are presented through three tables, which are quoted as pictures below (figure 6, 7 & 8), while there is little elaboration in the main text.

Serial No.	Parameter	ZFN	TALEN	CRISPER/Cas	Reference
1	design simplicity	moderate (ZFNs need customized protein for every DNA sequence)	slightly complex (identical repeats are multiple, which creates technical issues of engineering and delivery into cells)	simpler (available versions for crRNA can be easily designed)	48
2	engineering feasibility	low	higher	highest	24,49
3	multiplex genome editing	few models	few models	high-yield multiplexing available (no need for obtaining embryonic stem cells)	48,50
4	large-scale library preparation	not much progress (need individual gene tailoring)	not much progress (need individual gene tailoring)	progress demonstrated (CRISPR only requires plasmid containing small oligonucleotides)	51
5	specificity	low	higher	highest	24
6	efficiency	normal[a]	normal[b]	high	24,48,52
7	cost	low	high	low	53

[a]Some new versions are more efficient[24,48] but CRISPR science is evolving more.
[b]Cpf1 protein addition will probably improve cell delivery methods.[51,52]

Figure 6: Biotechnology differences among Prototype Genome editing techniques (original article's table quotation) [21]

As previously documented, the design issues and engineering capacities show up as crucial determinants. Multiplexing capacity, simplicity in design and cost are seriously taken into

consideration, rendering CRIPSR a winner. What is interesting in this particular table is the use of the term “normal”, which is reserved for the parameter of efficiency for ZNF and TALEN. As long as there is no fixed efficient level that can be considered 'normal', the use of such a term seems rather comparative. Specificity, in this table shows up as “low, higher, highest” while in previous articles, it has been described in quite the opposite direction (e.g. in 2017 article CRISPR has been characterized as a low specificity system).

Serial No.	Parameter	ZFN	TALEN	CRISPER/Cas	Reference
1	off-target effect incidence	–	–	–	54
a	homologous recombination rate frequency	+	+	+	–
b	non-homologous end joining (NHEJ) mutation rates	+	+	++ (only with earlier versions)	55,56
c	immune reaction susceptibility	less	less	more	57,58
d	RNA-guided endonuclease (RGEN)-induced off-target mutatagenesis	–	–	++	59
2	cytotoxicity chances	++	+	+	–

Figure 7: Side effects Profiles for Genome editing techniques (original article's table quotation)

In the table that examines the safety profile of each technique, the use of “-” indicating lack of data is present on maybe the most important parameter: the off-target incidences. The parameter of RGEN off-target mutagenesis is also mentioned, even if only CRISPR can be mentioned in this parameter (as the only RNA-guided endonuclease – the other two are protein-guided). Also, CRISPR is presented as more likely to raise immunogenic responses than the other two. ZNF is presented as being the most likely to provoke cytotoxicity only in the cytotoxic chances. One can be reassured that the safety profile is a rather blurry aspect of gene editing and that CRISPR does not seem to be the best option.

Serial No.	Parameter	ZFN	TALEN	CRISPER/Cas	Reference
1	diagnostic utility	+	+	+++	60
2	clinical trial use	++	+	+++	61
3	utility as epigenetic marker	++	+++	++++	62
4	making gene-knockout models for research	no	no	yes (CRISPRi)	63
5	capacity for modification of mitochondrial DNA	no	no	probable	64
6	genetic editing in human babies	no	no	yes	65
7	RNA editing	no	no	yes	66

Figure 8: Clinical and Research Applications across Important Genome-Editing Techniques (original article's table quotation)

On the third table, where for the purposes of comparison the applications are taken into consideration, CRISPR is the only technique that can be used as diagnostic tool in clinical trials, in epigenetic marker, in new animal model generation, in RNA editing and in modifying human embryos. In this rather 'all-in-one' presentation of applications that ranges from animal models to human embryos, CRISPR seems to be the most capable tool.

At the end of the article, the vision of genome re-creation is also mentioned. It is stated that one of the most interesting features of this technology is the possibility of synthesizing a genome from scratch, which will lead said technology to dominate the field of synthetic biology. The authors also mention the ethical issues that gene editing entails and state that although time will show if these technologies are "boon or bane", "still the methods can impact the human race probably in the most nuclear ways, and our incoming human race may be victimized in ways we do not yet understand." [21 p332].

The last paper of the primary literature results presentation was published in 2019 [56]. Although the article's primary subject concerns the delivery methods of ZNF, TALEN and CRISPR in the cells, a brief comparison of the three molecules' general performance is also conducted. In the article's main text it is stated that "[b]ecause the DNA binding specificity of TALEs is easier to engineer than zinc-finger proteins, TALEs can be applied more widely to life sciences than ZFN"[56 p735] , while below this we find the claim that "[b]ecause of the simplicity and high efficiency of the CRISPR-Cas9 system, Cas9 nuclease engineering has expanded to further applications" [56 p736]. Prioritizing simplicity, specificity and mode of engineering, they grade CRISPR first, TALEN second and ZNF

last. As in previous articles, the basic comparison parameters are also presented in a table (figure 9).

	Meganuclease	ZFN	TALEN	Cas9
Target sequence (bp)	14–40	9–18	14–20	~23
Number of target sites	limited	many	many	many
Enzyme engineering	difficult	difficult	easy	very easy
Size (kb)	~1	~1	~3	~3.5–4.5
Target recognition	protein-DNA	protein-DNA	protein-DNA	RNA-DNA base pairing and protein-DNA

Figure 9: Programmable Nuclease in Genome Editing (original article's table quotation)[56]

Although in the majority of the parameters presented only a simple data reference is made (meaning that the best option depends either on the expert's judgment or the system of employment), in certain parameters (like enzyme engineering and number of targets) a more fundamental categorization norms appear. Specifically, while the parameters "size of target sequence", "size" and "target recognition" are mostly expressed in numbers, the parameters "number of target sites" and "enzyme engineering" are compared verbally. It seems as if the authors are carefully (or impulsively) expressing a judgment concerning these two specific parameters, which along with all the others assert Cas-9 as the preferable tool. On the article's conclusions, the HIV clinical trials are mentioned again (along with the biotech companies that have them on track) and, once more, they state the fact that CRISPR-Cas9's "robust and high efficiency has transformed many areas of research in biomedicine".

4.4. Summary table – the terms of comparison

For reasons of convenience, a table summarizing the basic norms and terms of comparison that appeared in the primary literature is presented below. If a general classification regarding the parameters of comparison would be appropriate, then safety factors would be measured with cytotoxicity, off-target effects, immune reactivity and method of delivery; specificity, time, cost, programming capability (design) and multiplexing pertain to engineering and, therefore, to efficacy features. The basic terminology has been added in the Glossary. For the cases where it was provided, the general conclusion regarding the 'best' technique is also included.

Table 1: Means of comparison and terms of measurements

Year of publication	Authors	Technique under review	Safety parameters	Efficiency parameters	Conclusions over the "best" technique
2005 / Nature Biotechnology	M. H. Porteus and D. Carroll,	ZNF	-Toxicity -Off-target effects -Delivery methods	-Transformation - Efficiency	-
2008/Molecular therapy	T. Cathomen and J. Keith Joung,	ZNF	-DNA-binding specificity -Efficient delivery -Transient ZFN expression -Immune reactivity	-Transformation - Eficiency -Temporal and tissue specific gene expression	-
2010/trends in biotechnology	F. Le Provost, S. Lillico, B. Passet, R. Young, B. Whitelaw, and J. L. Vilotte	ZNF	-Off-target effects	-Efficiency -Engineering method	ZNF is a more "attractive, faster, and perhaps less challenging alternative"
2010/Nature Reviews	F. D. Urnov, E. J. Rebar, M. C. Holmes, H. S. Zhang, and P. D. Gregory	ZNF	-Off-target effects	-Versatility -Specificity -Simplicity -Engineering platform -Persistent -Fully penetrant	"ZFN-mediated genome editing now offers the ability to carry out sophisticated gene-function studies directly in the model system of interest"
2012/ Molecular Therapy–Nucleic Acids	Daniel F Carlson, Scott C Fahrenkrug and Perry B Hackett	ZNF + TALEN	-Cytotoxicity -Immunogenicity -Means of delivery	-Engineering design -Failure rates -Labor -Time -Transfection efficiency	"[W]e expect increasing adoption of TALENs by the research community. This bodes well for the development of new genetic models and effective

				-Specificity	therapies for our most prevalent congenital diseases."
2013/ Trends in biotechnology	T. Gaj, C. A. Gersbach, and C. F. Barbas,	ZNF + TALEN +CRISPR	-Mutagenesis -Toxicity	-Specificity -Engineering potential -Efficiency -Multiplexing -Ease of application	"ZFNs and TALENs are capable of correcting the underlying cause of the disease, therefore permanently eliminating the symptoms with precise genome modifications"
2014/Nature reviews	H. Kim and J. S. Kim	ZNF +TALEN + CRISPR	-Mutation signature -Off-target effects -Cytotoxicity -Delivery method	-Specificity, -Multiplex, -Time -Design -Construct -Availability -Composition -Targetable sites	-
2015/Nature Medicine	D. B. T. Cox, R. J. Platt, and F. Zhang,	ZNF +TALEN + CRISPR	-Immunogenicity -Delivery	-Specificity -Targeting constrains -Ease of engineering -Multiplexing	-
2016/ Molecular Therapy	M. L. Maeder and C. A. Gersbach	ZNF +TALEN + CRISPR	-Off-target -Immunogenicity -Biocompatibility of the carrier -Delivery method	-Specificity -Labor -Engineering potential	-
2016/Trends in Biotechnology	C. N. Kanchiswamy, M. Maffei, M. Malnoy, R. Velasco, and J. S. Kim	ZNF +TALEN + CRISPR	-Off-target -Cytotoxicity	-Ease of application -Multiplexing -Large scale libraries generation -Time -Labor	"GE tools including CRISPR-Cas9 have revolutionized genome engineering, and a major goal is to develop therapeutic applications of GE tools, particularly CRISPR-Cas9, to treat and cure genetic human and animal diseases or to use them to modulate novel traits in agriculture"

2016/Nature Protocol-Perspective	J. S. Kim	ZNF +TALEN + CRISPR	-Cytotoxicity -Off-target	-Engineering -Specificity	"Whereas CRISPR–Cas9 is likely to remain the tool of choice in academia, both ZFNs and TALENs will find roles in future applications and innovations: to date, both ZFNs and TALENs have been successfully used in human clinical trials"
2017/Nature Medicine	T. I. Cornu, C. Mussolino, and T. Cathomen	ZNF +TALEN + CRISPR	-Off-target activity -Mutagenesis -Differentiation in fitness and cell viability	-Specificity -Targeting range	-
2018/Trends in Genetics	R. O. Bak, N. Gomez-Ospina, and M. H. Porteus	ZNF +TALEN + CRISPR	-Toxicity -Off-target	-Speed -Simplicity -Cost -Specificity	The democratization and widespread use of genome editing was enabled by the discovery of the clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 nuclease system
2019/Molecular Therapy	H. X. Zhang, Y. Zhang, and H. Yin	ZNF +TALEN + CRISPR	-Cytotoxicity -Immune reaction -Delivery method	-Engineering -Simplicity -Efficiency -Size -Target recognition	Importantly, the breakthrough discovery of CRISPR-Cas9 genome-editing nucleases and their robust and high efficiency in precisely editing genome DNA has transformed many areas of research in biomedicine and has spurred RNA-based delivery to facilitate clinical translation
2019/Molecular therapy – Nucleic acids	S. H. Khan	ZNF +TALEN + CRISPR	-Cytotoxicity -Immune reaction	-Better efficiency -Feasibility and multi-role clinical application -Multiplexing capacity -Simplicity in design -Cost	"The latest discovery of CRISPR/Cas9 technology seems more encouraging by providing better efficiency, feasibility, and multi-role clinical application."

5. Discussion

Before getting to the last part of this thesis, it is important to state that neither the truth nor the unanimity of these scientific results are in question. What this thesis tries to understand is the social processes that drive gene editing as a scientific practice and, also, evaluate how they may shape our world. For this purpose, a literature search for the time-frame 2005-2019 has been conducted on two of the most prominent scientific journals, *Cell* and *Nature*. The search was conducted in their respective websites' search tool was and only review articles were included. The combination of the keywords “ZNF”, “TALEN” and “CRISPR” was submitted and a secondary review on each article's abstract was conducted, in order to include the articles that focus on the comparison of the three techniques. Textual analysis of the scientific literature has been utilized as the basic methodology, so as to take into consideration the narrative and the terms of comparison. This analysis, through an interpretation that is grounded on STS's literature (section 2), may provide some strong insights with respect to the social character of what is considered as an objective and rightful process of knowledge accumulation.

5.1. The narrative of progress

Based on the analysis of the primary literature, it became obvious that gene editing is depicted as a progressive process that leads scientific community to better results. Gene editing is framed through terms like “powerful tools”, “control and accuracy”, “challenges and advances”, “revolution”, “exciting tools”, “landscape changing” and “inevitable”, which is typical of the enthusiasm that pervades the field. This sense of power and the perception of numerous capabilities has been present from the very beginning, when ZNF was the only technique constructed and tested.

Another important feature of the literature's narrative is the absence of definite negative results. Although difficulties and limitations are mentioned for each one of them, they are often labeled as challenges and only become restrains and weaknesses after the emergence of the next technique. Low efficiency rates, cytotoxic results or, in general, failed experiments are occupying very few lines in the literature and are mostly attributed to intrinsic characteristics of the technique that need to be fixed. For example, in the case of ZNF, the parameters of cytotoxicity and off-target activity are initially mentioned as things that need to be taken into consideration for future prospects. Yet, in the 2012 paper the same parameters are presented as problems that can be solved by TALENS. The whole

process of succession is inspired by the motion of ongoing progress and every limitation is reducible to the technique per se and not on the experiments' condition.

In general, a very strong but silent assumption permeates the primary literature and is in accordance with the centralization of techniques and the narrative of progress. The fact that each technique is presented ‘in vacuum' is very striking. The authors' assumptions and expectations regarding the probabilistic character of how this is expected to work are almost interchangeable with the experimental data that support these representations. Sentences like: “**must** exhibit an extraordinary combination of qualities”, “ZFNs, combined with a single donor DNA, **can be** used for 'correcting' all mutations”, “Gene disruption by NHEJ at a target gene **can** be a definitive method to investigate gene function” are some examples of this general tendency to theorize the available data according to their expected function. Moreover –and, maybe, more importantly– although it is clearly stated that gene editing is highly cell- and system-dependent, these parameters are only analyzed as variables that need to be fixed in order for the technique to function better. The description of ZNF, TALEN and CRISPR's functionality which is present in every article emerges as a general rule. The detailed description of how each molecule works is stated as a common process regardless of the type of cell or organism, the mode of delivery, the stage of the cell, the specific difficulties and errors. Gene editing arises as a totally context-independent mechanism where all the other components of the procedure are regarded as proper adjustments that shall be made in order for the technique to fit all different purposes. As Shapin and Medawar have commented, the scientific narrative is characterized by practices that tend to treat the given representations as general rules and objects that already exist. The use of the passive voice and the probabilistic nature of the detailed descriptions of the experimental arrays are some of the characteristics that hold true for the case in question, as well.

As dictated by the majority of scientific journals, an article needs to meet a specific structure format as a publishing prerequisite. This usually allocates relevant scientific data to the first part and possible applications to the second, with a narrative of presumptive causal connection between data and application following them. This is the case for all of the articles in the primary literature. In the first part of the article, gene editing's general mechanism is presented and each molecule's performance is articulated as the basic phenomenon upon which different applications are staged. This is usually expounded in the second part of the article, where future or current clinical applicability is examined. Since the earliest article only dealt with ZNF, in its first part different aspects of how this technique is supposed to work are detailed (deletion, insertion, correction) along with the molecule's specificity and mode of engineering. Similarly, when the other two techniques came handy, the first part is

typically concerned with the possible mechanisms and the progress in the modes of use, the different engineering platforms and the comparison between them. In the second part, the implementation of techniques over different systems and, more specifically, its clinical use is usually described. In this kind of apposition, though, a different succession comes as a rational next step: the succession from research animals and in vitro cell lines to the human body.

By 2005 there were almost no clinical trials, while few were completed by 2020. Although this restricts the reference to results from clinical applications, almost every article presents gene editing as a powerful tool able to transform the field of therapy. The fact that there is poor differentiation between the data that result from animal testing, in vitro cell lines or human sources –along with the above-mentioned 'in vacuum' narrative– contributes to a general sense that there is no long road from bench to bedside anymore. In fact, in the more recent literature, CRISPR-Cas9 has been characterized as having "transformed many areas of research in biomedicine" – a perspective that is also clearly stated in the 2018 paper, when the author comments that "it is only by testing them in the clinic that we will learn about the actual problems, expected or unexpected, and begin working on solving them". It appears that gene editing progresses as an ever-improving method to the very deliberate direction of human application due to its decisive and powerful character.

5.2. The norms of progress

By coming closer to the norms upon which the field is progressing, we have already encountered the notion of a 'paradigm'. Perhaps Kuhn would have been somewhat surprised if he knew how often the 'paradigm' terminology would be put to use in technical scientific papers. Phrases like "site-specific nucleases for therapeutic purposes represents a paradigm shift in gene therapy" have shown up in some of the articles presented above. So, is it then true that gene editing is a paradigm shifter? Having a thorough look at the basic determinants of comparison, we can observe that these are almost the same since 2005 (Table 1). The comparison between the techniques is mostly based on the following parameters: specificity, efficacy, off-target effects, cytotoxicity, immune reactivity, time, cost, multiplexing, ease of design and method of delivery.

A very reasonable assumption (and my initial one) would be to assume that CRISPR is the safest and most efficient one, given the great applicability and popularity it has gained since its introduction to the field. This, however, seems to not be entirely accurate. The facts regarding the so-called safety determinants (e.g. off-target effects that are correlated with undesired DNA breaks which ultimately

may lead to unpredictable and mutagenic events, cytotoxicity that may lead to cell death or uncontrollable propagation, immune reactivity that may lead to an immune system attack exacerbation and generalized breakdown) are still at a rudimentary stage. On the first papers of the analysis, cytotoxicity and off-target effects are mentioned as points that should be better understood before ZNF moves to the clinic. Later on, the discussion becomes concerned with the available methods of measuring the off-target effects. The most recent articles show that little progress has been achieved regarding any 'definite' answers about gene editing's safety. Similar conclusions may be extracted from the 'condensed' tables that summarize the comparison features. When defining the off-target effects parameter, the sign of "-'" (for unknown) or the phrase "low to moderate" are frequently showing up, while, concerning the parameter of immune reactivity, CRISPR has been assigned the status of "more" or "unknown". The specificity parameters produce similar results. For example, CRISPR has been characterized as having low specificity in the 2015 and 2017 articles (compared to previous methods), while in a 2016 and a 2019 article it is characterized as the most specific one. There may be numerous reasons behind the diversity in the basic determinants' results, yet, once again, none of the specific references are questioned. What is at stake here, though, is a viable pushback to the definite narrative of success and progress that surrounds CRISPR, by advancing the concerns of the scientific community regarding its safety and its efficacy. The form of table, for purposes of comparison summarizing, is reinforcing also the 'generality' of the truth these tables hold and are in accordance with the narrative of progress. But if someone would put all this information together, then confusion regarding each molecule's safety and specificity would arise. Different meaning attribution to each parameter or different systems of application can probably help to better situate each molecule.

So, if CRISPR is not the safest technique or –more accurately– if the scientific community has not yet reached safe conclusions about the way these molecules perform and the proper method of these parameters' measurement, then why has CRISPR been so highly valued by scientists? On which grounds is CRISPR promoted over its predecessors? Maybe diversity is a given for safety factors, but regarding CRISPR's programmability there is an almost unanimous answer: it's easy. As presented above, different engineering parameters are taken into consideration regarding the techniques' comparison. Cost, time, multiplexing, re-targeting programmability, design, large scale libraries generation are some of the most important parameters that show up in almost every article. CRISPR as an RNA guide molecule is the 'winner' in every one of these determinants. It has been characterized as "simple", "multirole", "high yield multiplexing", "easy" and "low cost", while ZNF and TALEN (which are protein guided) have been characterized as "time consuming", "laborious", "complex"

and “difficult”. The word “platform” is also extensively used when the discussion comes to the most appropriate mode of programming. The different programming strategies are covering a very big portion of almost every article, revealing their great importance for the scientific community. It is also striking that the word “ease” is incorporated into the parameters column of two different comparison tables. It is more than obvious that the scientific community promotes the use of CRISPR because of engineering reasons.

The political economy of each molecule's design is the leading determinant and, as expected, the least costly and, crucially, the most time-saving will be the first option. As previously analyzed, epistemological dominance is not a socio-politically independent process. A method, just like an artifact, is discussed and comprehended in terms already embedded in our perception. Therefore, an RNA-guided molecule would seem like a more reasonable choice over a protein molecule. After many years of intensive research, RNA got easier to handle and more familiar to us. Therefore, it is assumed to be offering greater programmability possibilities and a modular engineering that may allow for a more 'platform'-centralized trajectory. As stated before, “for better or worse” CRISPR dominates the field because of its ability to intensify the experimentation process on the clinical level. As with the metal and wooden airplanes in Schaltzberg's analysis, progress is once more proven to not emanate from the 'rational' dominance of what may be perceived as a better method or technique.

5.3. The vision of progress

At first glance, one expectation pervades almost every article: the vision of therapy. Having as a background other gene therapy technique where some really serious adverse effects were developed, gene editing again filled the scientific community with expectations regarding gene therapy's feasibility. CRISPR has been regarded as the technique with the broadest applications (from a diagnostic tool to clinical trials, an epigenetic marker and genome modification). Sentences like “there are many reasons to be optimistic that soon an entirely new class of genetic therapeutics based on genome editing will be available to a wide range of patients”, “ the ZFN strategy may be applied in the treatment of human genetic diseases”, “similarity of the concepts of gene therapy and transgenesis will lead to further technological improvements”, “ZFNs and TALENs are capable of correcting the underlying cause of the disease” along with the –previously analyzed– frequently met structure, contribute to the general sense that gene editing is a strategy which is clearly oriented to the human body. This vision is not a new one as, ever since the rRNA period, the concept of gene therapy has been evolving. In more recent years this vision has been transformed into a purpose and,

nowadays, gene editing seems to be leading the research field. Besides the conceptualization of therapy, other visions seem to have also been formed around gene editing. As already mentioned, in a 2019 article gene editing is expected to play a major role in synthetic biology by providing the capability of resynthesizing the whole genome, while in a 2016 article a more bold expectation regarding the possibilities of gene editing is articulated: "the programmable nucleases, may eventually enable humans—products of evolution—to become **masters of evolution".** So, what are the contingent scenarios that are enabled from gene editing and how distinct are the three visions found in this primary literature?

Looking at the literature more carefully, we come across another interesting term: the concept of reverse genetics. As pointed out in three articles, gene editing is the epistemological conceptualization of reverse genetics. Reverse genetics is based on the norm that genes come first in the causal relationship between genotype and phenotype. This viewpoint has also staged genetic determinism as a research methodology. Preceding this concept, in classic genetics phenotype and, therefore, proteins were more central for disease causality and the therapeutic approach. The strategy of protein augmentation has been used successfully in diseases like cystic fibrosis. Even diseases that are now widely tested for gene editing therapy (like sickle cell anemia) were fairly treated via a protein therapy approach. "Before the development of genetic medicines, strategies for treating hereditary disorders focused on metabolic manipulation and protein augmentation therapy. For some monogenic disorders, success with these approaches has been remarkable" [57]. Putting proteins or metabolism first (although this might seem like a small difference) creates a wholly different context. Protein-based therapy may be diet dependent (like in the case of phenylketonuria) or more-human-physiology dependent (like in the case of human metabolic diseases). Most of all, it would require a wholly different research field. In the rise of genetic medicine, though, the boundaries between different systems (of course, only on a lab research level) are lost in the big pool of genes that are common or reserved between various model organisms and the research approach of trying to understand what would happen in the phenotype if a gene that has been changed prevails. Years of experimentation on this path have really distanced us from better understanding physiology and metabolism processes. On the other side, gene editing comes as the mean to achieve reverse genetics in the clinic. The vision of correcting a gene in order to fix the phenotype is once again reaching the clinic. Yet, this time it is on the shoulders of CRISPR. But for which cases can reverse genetics serve as a therapy strategy?

Although monogenic diseases (diseases that are attributed to a gene abnormality) have been the initial clinical target of ZNF and TALEN, CRISPR has widened this goal to almost every disease that might

depend upon genetic factors (like cancer and HIV). Multiplexing is one of CRISPR's primary features that may help with this strategy and this probably is why this technique is mostly sought after. The epistemological domination of reverse genetics is a common ground for the majority of in vivo lab research, but its clinical introduction has been a more recent venture. From a casual perspective, before therapy comes disease and the field is not at all invested in defining the latter, as is tacitly admitted by the technique's affordances. If gene editing offers the possibility of therapy, then what is a cure about and how does it happen?

5.4. The right cure requires the right disease

Having this last question in mind, different features arise. As stated before, although in the initial level only monogenic diseases have been the primary target of gene editing, later on, more complex diseases like cancer (which is far from monogenic) have been tested. In the attempt to understand what is gene therapy possible to cure and how this is shaping the field of its clinical applications, the technique's affordances are rising as the basic determinant over what can be cured and, therefore, what can be conceived as disease. Specific characteristics need to be met at this stage in order for gene editing to have better chances of achieving therapy. These characteristics are mostly gathered as desired features on the treated cells. As already described, the engineering platform may be the central concern of all these articles, but the treated cells are the human therapy's unit and, therefore, the desirable features are formed as desired cellular characteristics. Gene editing contributes to the reintroduction of cells in the human body as a technology. Human cell lines and, in general, cells have a very varied story that forms a circle with gene therapy. Cells were isolated, engineered and then reintroduced or directly engineered, reaching the human body not as a cell anymore, but as gene editing technology's therapy unit. Spanning the field from genes to cells, these need to acquire specific characteristics according to the literature. The most desired cells are those that can still propagate, easily reach the diseased target, repopulate it and stay there for as long as possible. However, cell cycle life and human physiology might displace the success story of engineered features into more challenging trajectories. Another feature that is considered an essential one is “fitness”. As explained by the 2015 article, gene editing's therapy may be optimally applied in “diseases in which edited cells possess a fitness advantage, so that a small number of engrafted, edited cells can expand and treat disease. One such disease is HIV, as HIV infection results in a fitness disadvantage to CD4+ T cells”. This explains why HIV and hematological diseases are the most appropriate ‘human disease models'. If research needs to extend to the human body then, based on the already known approach, disease models will be needed. Indeed, HIV has served as the most frequently met disease in gene editing's

clinical trials. Here, a previously highlighted statement is quire fitting: “if conventional therapies are already available, such as treatment of HIV infection, a gene-editing approach in hematopoietic stem cells in combination with a cytotoxic-conditioning regime must persuasively demonstrate both the safety of the manufactured stem cell product and its potential to achieve a level of benefit that exceeds that provided by current treatment options”. Thus, gene therapy may still be perceived as therapy even if there are alternative therapies, on the basis of higher quality treatment.

Successful therapy is then dictated by the disease in question. What seems to be at stake when gene editing reaches the clinic is not so much the therapeutic norms, but the ones concerning the disease. Through gene editing and in consistence with the norm of reverse genetics, diseases seem to be conceived as the probabilistic state of one or multiple bad genes expression resulting in wrong phenotypes. Summing up the desired features, engineered cells need to be in the proliferation phase, which means they need to be in a younger or less differentiated stage, have better fitness in order to dominate the desired organ or organism and avoid mosaicism effects. Also, they should have a prolonged and time lasting effect. Putting it bluntly, gene editing would have its best performance and chance to succeed if it is applied on an embryo. If this therapeutic approach, this disease conception and the techniques' affordances are 'aligned', then Nana and Lulu may be deemed as the best way to have successful results in a race where the visions of human therapy and mastery of evolution are remarkably intertwined.

5.5. Concluding remarks

This thesis has been concerned with a very specific facet of gene editing: how is this field staged and depicted in the scientific literature and what are the basic axes of comparison between the three techniques that comprise it. In an attempt to discern in what ways CRISPR has revolutionized the field, some sociotechnical insights were gained by scrutinizing the central narrative and parameters. Indeed, the literature around gene editing has been imbued with very intense and cheerful emotions, which seem to be mostly inspired by the possibility of performing reverse genetics in the clinic. Specifically, CRISPR emerges as the ‘favored' tool, as it has brought bench and bedside closer than ever. Its easy, simple, less laborious and multiplex design seems to enable the intensification of experimentation in the field of gene modification. The vision of correcting 'bad' genes has been transformed into a broad therapeutic strategy that consists in the exploration and treatment of the combinational expression of genetic factors that may contribute to a disease. Far from the neutral and

objective character that science is supposed to have, specific norms appear to lead to practices that propagate what is already embedded in modern economy. Put more dramatically, these practices generate realities according to which the human body has to conform to a method's efficacy. Sheila Jasanoff (et al.) has commented that "[s]cience and technology not only improve lives but shape our expectations, and eventually our experiences, of how lives ought to be lived" [31 p]. If disease is perceived as a painful state which can be reduced to definite countable units (genes), then therapy emerges as the rational treatment of probable genetic expressions. And this is neither neutral nor objective.

6. References

[1] R S Brown, C Sander, P Argos. The primary structure of transcription factor TFIIIA has 12 consecutive repeats. FEBS Lett. 1985 vol186(2):271-4. doi: 10.1016/0014-5793(85)80723-7.

[2] J Miller, A D McLachlan, A Klug Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. EMBO. 1985, vol 4(6):1609-14.

[3] Bonas U, Stall RE, Staskawicz. Genetic and structural characterization of the avirulence gene avrBs3 from Xanthomonas campestris pv. Vesicatoria. B. Mol. Gen. Genet. 1989; 218:127

[4] F.J.M. Mojica, G. Juez, F. Rodríguez-Valera.Transcription at different salinities of Haloferax mediterranei sequences adjacent to partially modified PstI sites Mol. Microbiol., 9 (1993), pp. 613-621

[5] Morrison, M., de Saille, S. CRISPR in context: towards a socially responsible debate on embryo editing. Palgrave Commun 5, 2019, 110. https://doi.org/10.1057/s41599-019-0319-5

[6] Cohen, S.; Chang, A.; Boyer, H.; Helling, R. Construction of biologically functional bacterial plasmids in vitro. Proceedings of the National Academy of Sciences of the United States of America. 1973,0 (11): 3240–3244. doi:10.1073/pnas.70.11.3240. PMC 427208. PMID 4594039.

[7] Gerry Waneck. Safety and Health issues revisited, Science for People, 1985, vol. 17, no. 3,

[8] Rosemary Robins, The realness of risk. Soc. Stud. Sci., 2002, vol. 32, no. 1, pp. 7–35,.

[9] E. Smalley, As CRISPR – Cas adoption soars , summit calls for genome editing oversight,Nat. Publ. Gr., 2018, vol. 36, no. 6, p. 485, doi: 10.1038/nbt0618-485.

[10] National Academies of Sciences, Engineering, and Medicine; Policy and Global Affairs. Second International Summit on Human Genome Editing: Continuing the Global Discussion: Proceedings of a Workshop—in Brief. Washington (DC): National Academies Press (US); 2019 Jan 10. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535994/ doi: 10.17226/25343

[11] F. D. Urnov, Genome Editing B . C . (Before CRISPR): Lasting Lessons from the ‘“ Old Testament ,” 2018, vol. 1, no. 1, pp. 34–46, , doi: 10.1089/crispr.2018.29007.fyu.

[12] S. K. Saha et al., Programmable Molecular Scissors: Applications of a New Tool for Genome Editing in Biotech, Mol. Ther. - Nucleic Acids, 2019, vol. 14, no. March, pp. 212–238, doi: 10.1016/j.omtn.2018.11.016.

[13] Martin Jinek, Krzysztof Chylinski, Ines Fonfara, Michael Hauer, Jennifer A.Doudna, and Emmanuelle Charpentier, A programmable dual RNA-guided DNA endonuclease in adaptive bacterial immunity, Science. 2012 vol 337(6096): 816–821. Published online 2012 Jun 28. doi: 10.1126/science.1225829

[14] Maria Jasin1 and James E. Haber2 The Democratization of Gene Editing: Insights from site-

specific cleavage and double-strand break repair, DNA Repair (Amst). 2016 vol44: 6–16. Published online 2016 May 12. doi: 10.1016/j.dnarep.2016.05.001
[15] Wikipedia- Jennifer Doudna
[16] H. Li, Y. Yang, W. Hong, M. Huang, M. Wu, and X. Zhao, Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects, Signal Transduct. Target. Ther., 2019, doi: 10.1038/s41392-019-0089-y.
[17] T. Gaj, C. A. Gersbach, and C. F. Barbas, ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering, Trends Biotechnol., 2013, vol. 31, no. 7, pp. 397–405, doi10.1016/j.tibtech.2013.04.004.
[18] Method of the Year 2011. Nat Methods 9, 1 (2012). https://doi.org/10.1038/nmeth.1852
[19] Science News Staff . And Science's 2015 Breakthrough of the Year is...URL: https://www.sciencemag.org/news/2015/12/and-science-s-2015-breakthrough-year., 2015
[20] K. Brinegar, A. K. Yetisen, S. Choi, E. Vallillo, and G. U. Ruiz-esparza, The commercialization of genome-editing technologies, CRITICAL REVIEWS IN BIOTECHNOLOGY, 2017, vol. 37, no. 7, pp. 924–932.
[21] S. H. Khan, Genome-Editing Technologies: Concept, Pros , and Cons of Various Genome-Editing Techniques and Bioethical Concerns for Clinical Application, Molecular Therapy: Nucleic Acids 2019,vol. 16, no. June, pp. 326–334, doi: 10.1016/j.omtn.2019.02.027.
[22] Urnov FD, Miller JC, Lee YL, et al. Highly efficient endoge-nous human gene correction using designed zinc-finger nucle-ases. Nature 2005; 435:646-651
[23] Cribbs AP, Perera SMW. Science and Bioethics of CRISPR-Cas9 Gene Editing: An Analysis Towards Separating Facts and Fiction. Yale J Biol Med. 2017;90(4):625-634. Published 2017 Dec 19. 4.
[24] Li JR, Walker S, Nie JB, Zhang XQ. Experiments that led to the first gene-edited babies: the ethical failings and the urgent need for better governance. J Zhejiang Univ Sci B. 2019;20(1):32-38. doi:10.1631/jzus.B1800624.
[25] S. Sismondo, An Introduction to Science and Technology Studies Second Edition. Wiley-Blackwell,2011
[26] Bird, Alexander, Thomas Kuhn, The Stanford Encyclopedia of Philosophy, 2018 Edition, Edward N. Zalta (ed.), URL<https://plato.stanford.edu/archives/win2018/entries/thomas-kuhn/>.,
[27] Eric Schaltzberg, Ideology and Technical Choice: The decline of eooden airplane in the United States, 1920 - 1945, 1994, pp. 200–201,.
[28] Steven Shapin, Pump and Circumstance: Robert Boyle ' s Literary Technology, Social Studies of Science , 1984, Vol .4 , No .4 pp .481-520 URL : http://www.jstor.org/stable," vol. 14, no. 4, pp.

481–520, 2016.
[29] Medawar P. Is the scientific paper a fraud? Listener. 1963 vol70:377–378.
[30] Alyssa Botelho, The Insights of Radical Science in the CRISPR Gene-Editing Era: A History of Science for the People and the Cambridge Recombinant DNA Controversy, Sci. Cult. (Lond)., 2019.
[31] S. Jasanoff, J. B. Hurlbut, and K. Saha, CRISPR Democracy: Gene Editing and the Need for Inclusive Deliberation, Issues in Science and technology,2015 vol. XXXII, no. 1.
[32] Montenegro de Wit, M. Democratizing CRISPR? Stories, practices, and politics of science and governance on the agricultural gene editing frontier. Elem Sci Anth, 8(1), p.9. 2020, DOI: http://doi.org/10.1525/elementa.405.
[33] G. of I. Department of Biotechnology, Draft Document on Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment Draft Document on Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment Draft Document on Genome Edited Organisms: Regulatory Fra, pp. 3–42, 2020.
[34] U. of O. Søren Holm, Let us assume that gene editing is safe – the role of safety arguments in the gene editing debate, Centre for Social Ethics and Policy, School of Law, University of Manchester, Manchester M13 9PL, UK & Centre for Medical Ethics, HELSAM, 2018.
[35] Nuffield Council on Bioethics, 28 Bedford Square, and L. W. 3JS, “Genome editing,” 2016.
[36] A. Lang et al., A molecular scalpel for intervention in the genome: Opportunities and risks associated with genome editing.
[37] ETHICS COUNCIL OF THE MAX PLANCK SOCIETY, “Discussion paper focusing on the scientific relevance of genome editing and on the ethical, legal and societal issues potentially involved,” 2019.
[38] M. Braun and P. Dabrock, Wager on Gene Editing Techniques, EMBO Rep. vol. 17, no. 3, pp. 279–280, 2016, doi: 10.15252/embr.201541935.
[39] V. Marx, Gene editing: How to stay on-target with CRISPR, Nat. Methods, vol. 11, no. 10, pp. 1021–1026, 2014, doi: 10.1038/nmeth.3108.
[40] C. Publishing, CRISPR-Cas Revolution in Gene Editing,Science/AAAS, 2014.
[41] M. Morrison and S. de Saille, CRISPR in context: towards a socially responsible debate on embryo editing, Palgrave Commun., 2019, vol. 5, no. 1, pp. 1–9, doi: 10.1057/s41599-019-0319-5.
[42] E. Smalley, As CRISPR – Cas adoption soars, summit calls for genome editing oversight, Nat. Publ. Gr., 2018, vol. 36, no. 6, p. 485, , doi: 10.1038/nbt0618-485.
[43]M. H. Porteus and D. Carroll, Gene targeting using zinc finger nucleases, Nat. Biotechnol., 2005, vol. 23, no. 8, pp. 967–973, doi: 10.1038/nbt1125.

[44] T. Cathomen and J. Keith Joung, Zinc-finger nucleases: The next generation emerges, Mol. Ther., 2008, vol. 16, no. 7, pp. 1200–1207 , doi: 10.1038/mt.2008.114.
[45] F. Le Provost, S. Lillico, B. Passet, R. Young, B. Whitelaw, and J. L. Vilotte, Zinc finger nuclease technology heralds a new era in mammalian transgenesis, Trends Biotechnol., 2010, vol. 28, no. 3, pp. 134–141, doi: 10.1016/j.tibtech.2009.11.007.
[46] F. D. Urnov, E. J. Rebar, M. C. Holmes, H. S. Zhang, and P. D. Gregory, Genome editing with engineered zinc finger nucleases, Nat. Rev. Genet., 2010, vol. 11, no. 9, pp. 636–646, doi: 10.1038/nrg2842.
[47] D. F. Carlson, S. C. Fahrenkrug, and P. B. Hackett, Targeting DNA with fingers and TALENs, Mol. Ther. - Nucleic Acids, 2012 vol. 1, no. 1, p. e3,doi: 10.1038/mtna.2011.5.
[48] T. Gaj, C. A. Gersbach, and C. F. Barbas, ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering, Trends Biotechnol., 2013vol. 31, no. 7, pp. 397–405, doi: 10.1016/j.tibtech.2013.04.004.
[49] H. Kim and J. S. Kim, A guide to genome engineering with programmable nucleases, Nat. Rev. Genet., 2014, vol. 15, no. 5, pp. 321–334, doi: 10.1038/nrg3686.
[50] D. B. T. Cox, R. J. Platt, and F. Zhang, Therapeutic genome editing: Prospects and challenges, Nat. Med., 2015, vol. 21, no. 2, pp. 121–131, doi: 10.1038/nm.3793.
[51] M. L. Maeder and C. A. Gersbach, Genome-editing technologies for gene and cell therapy, Mol. Ther., 2016, vol. 24, no. 3, pp. 430–446, , doi: 10.1038/mt.2016.10.
[52] C. N. Kanchiswamy, M. Maffei, M. Malnoy, R. Velasco, and J. S. Kim, Fine-Tuning Next-Generation Genome Editing Tools, Trends Biotechnol., 2016, vol. 34, no. 7, pp. 562–574, , doi: 10.1016/j.tibtech.2016.03.007.
[53] J. S. Kim, Genome editing comes of age, Nat. Protoc., 2016, vol. 11, no. 9, pp. 1573–1578, doi: 10.1038/nprot.2016.104.
[54] T. I. Cornu, C. Mussolino, and T. Cathomen, Refining strategies to translate genome editing to the clinic, Nat. Med., 2017, vol. 23, no. 4, pp. 415–423, doi: 10.1038/nm.4313.
[55] R. O. Bak, N. Gomez-Ospina, and M. H. Porteus, Gene Editing on Center Stage, Trends Genet., 2018, vol. 34, no. 8, pp. 600–611, doi: 10.1016/j.tig.2018.05.004.
[56] H. X. Zhang, Y. Zhang, and H. Yin, Genome Editing with mRNA Encoding ZFN, TALEN, and Cas9, Mol. Ther., 2019, vol. 27, no. 4, pp. 735–746, , doi: 10.1016/j.ymthe.2019.01.014.
[57] T. P. O'Connor and R. G. Crystal, Genetic medicines: Treatment strategies for hereditary disorders, Nat. Rev. Genet., 2006, vol. 7, no. 4, pp. 261–276, , doi: 10.1038/nrg1829.
[58] CRISPR-Cas9 Tools – Global Market and Patent Landscape Report till 2025, Available from:

https://www.aheadintel.com/product/crispr-cas9-tools-global-market-and-patent-landscape-report-till-2025/

Appendix 1 – Abbreviations

CRISPR – Clustered regularly interspaced short palindromic repeats

Double strand breaks (DBS) – Breaks in both the DNA strands. Essential step in case of homologous recombination or DNA replacement.

TALEN – Transcription activator-like effector nucleases

ZNF – zinc-finger nucleases

Appendix 2 – Glossary

Biocompatibility – compatibility between the delivery vehicle and the recipient. Toxicity issues may arise in case of incompatibility

Cytotoxicity – the potential to provoke cell death

Differentiation – The potency of a cell to perform cell type specific functions. Less differentiated are “younger” cells (multipotent) that maintain the full genetic potency to commit to any cellular “fate”. Somatic cells are fully differentiated, “adult” cells that have the ability to perform specific functions that highly tissue dependent.

Ex vivo delivery – a method of cellular delivery that takes place outside of the body

Gene therapy – therapy strategy that target genes for disease cure or prevention

Homologous recombination – exchange between two similar or identical nucleotide sequences

Immune reactivity – the potency to provoke intense immunological response

In vivo delivery – a method of cellular delivery that takes place inside of the body

Large scale library preparation – Large collection of DNA fragments that have been cloned into vectors so that researchers can identify and isolate the DNA fragments that interest them for further study.

Mosaicism effects – a condition in which cells within the same person have a different genetic makeup

Multiplex – the ability to modify simultaneously several related or unrelated genomic sites targeted through delivery of one construct.

Monogenic disease – single gene occurring disease

Number of targets – a molecule's ability to target many or few genetic loci.

Non homologous end joining – a pathway that repairs double-strand breaks in DNA. It is used a basic repair pathway that will lead to a gene's mutation.

Nuclease – a molecule with enzymatic activity of cutting nucleotide sequences

Off target activity – potential to exert unintended activity. A term used to measure a molecule's ability to produce undesired effects and instability. For gene editing molecules, this means to provoke random DNA breaks.

Reverse genetics – An experimental and epistemological approach that was enabled with recombinant DAN and starts from a protein or DNA for which there is no genetic information and then works backward to make a mutant gene, ending up with a mutant phenotype.

Size of targets – the length (in nucleotides) that each molecule requires in order to attach.

Specificity – a molecule's ability to attach to a specific genetic locus with small or none mismatch. In case molecule can bind a genetic sequence while not being entirely complementary, it is considered as one with low specificity.

Targeting range – a molecule's ability to target few or many genetic locus based on the recognition pattern

Transfection efficiency – The rate of successful delivery and expression of a foreign molecule in a cell population