European Review for Medical and Pharmacological Sciences 2021; 25: 1752-1761

Commentary – CRISPR-based techniques: Cas9, Cas13 and their applications in the era of COVID-19

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Abstract. – The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 (CRISPR-associated protein 9) system enables scientists to edit diverse genome types with relative ease, with the aim – in the near future – to prevent future human beings from developing genetic diseases. The new opportunities arising from the system are broad-ranging and revolutionary, but such prospects have also been the cause for alarm throughout the international scientific community. The authors have laid out a review of the trials carried out so far in terms of genome editing, for the ultimate purpose of weighing implications and criticisms. We feel that possible valuable alternatives, such as induced pluripotent stem cells should not be overlooked.

Key Words:
CRISPR/Cas9-Cas13, Germline gene editing, Enhancement, Human embryo research, Sars-CoV-2.

Introduction

The New Frontiers of Biomedical Engineering

Genetic engineering saw the light in the 1960s, when it started helping scientists to figure out and, to a certain degree, control biological and pathological patterns, thus profoundly impacting medical science as a whole; most significantly it has contributed to the diagnosis and treatment of various diseases, as well as the development of new drugs. Mario Capecchi, co-winner of the 2007 Nobel Prize in Physiology or Medicine, mastered the technique of recombinant DNA (rDNA), demonstrating that an existing gene in mouse could be "knocked out" and replaced or disrupted with an artificial piece of DNA[1]. Later on, the opportunity to apply such techniques to create genetically modified animals was explored, by altering genes within zygotes (the cells from which embryos are originated)[2]. That became possible by means of genetic recombination, in which genetic information is exchanged between two similar or identical molecules of double-stranded or single-stranded nucleic acids (DNA as in cellular organisms, but also RNA in some viruses). Capecchi's research ultimately showed how the genes inserted into the cells positioned themselves randomly, but still followed the same orientation. That observation led him to assume the existence of a "mechanism" through which homologous recombination of DNA strands sharing the same sequence is brought about. Over time, he saw that such procedures could be harnessed to "knock out" a mutated gene (responsible for a disease) and replace it with a healthy one, and to edit genes at specific locations. In 1984, Capecchi, in collaboration with Martin Evans, learned how to produce and culture bioengineered embryonic cells to generate "knock-out" genetically modified mice[3]. The new era of gene targeting was dawning, which through techniques such as TALEN ("transcription activator-like effector nucleases"), restriction enzymes that can be engineered to cut specific sequences of DNA, and Zinc-finger nucleases (ZFNs) made it possible for scientists to alter the human genome at the embryonic level, triggering human genetic diseases in animal models *in vitro* to gain an insight into their development patterns and possible therapeutic options.

CRISPR/Cas9: An Innovative Tool in Embryonic Therapeutic Options

Research on germline editing took a decisive turn in 2012, the year CRISPR/Cas9 came to the fore. Specifically, the CRISPR/Cas9 system

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relies on a guide RNA (gRNA); these non-coding short RNA sequences bind to the complementary target DNA sequences. Guide RNA first binds to the Cas9 enzyme and the gRNA sequence guides the complex by pairing it to a specific location on the DNA, where Cas9 cuts the target DNA strand through its endonuclease activity[4]. It is worth pointing out that the CRISPR system is a natural system used by bacteria to protect themselves from bacteriophage infections. When the bacterium detects the presence of viral DNA encapsulated within its genome, it creates a short RNA sequence matching the DNA of the invading virus, which then gives rise to a complex response involving the Cas9 protein capable of excising DNA at a precise location. This system is akin to a sort of "molecular scissors", effective at removing the Viral DNA that matches the short RNA sequence, guided by the short bacterial RNA sequence Cas9, thus deactivating the virus. In light of its precision and efficiency, scientists began to search for modifications of the mechanism of Cas9, so that it could be utilized on any DNA, not just bacterial DNA, and more importantly, on living cells.

CRISPR carries a promise that could potentially revolutionize the medical field as we know it. Over 10,000 human diseases are caused by a single gene, and the CRISPR/Cas9 system could contribute to their eradication over time[5,6]. Specifically, recent findings have shown how CRISPR may be effective in correcting the mutation in a gene causing inherited heart disease in human embryos. Such a mutated gene (MYBPC3) was substituted by a "corrected" copy of the gene that did not carry the mutation. Ultimately, it was found that as many as 72% of embryos had been freed of the harmful mutation, with a high degree of accuracy and safety. Such data constitute the first conclusive proof to bear out the feasibility of the technology[7].

Still, plenty of unanswered questions and doubts linger about the technology itself. Accuracy is certainly a key point: how often does the desired mutation can reach and repair the targeted genome? Although the CRISPR/Cas9 system's targeting effectiveness has been deemed considerably higher than that of other gene-editing techniques, such as ZFNs and TALENs, that is partly due to the latter techniques' relatively low efficiency (ranging from 1 to 50%)[8,9] in trials with human cells. Although such levels of reliability are expected to improve as research goes forward, such targeting inaccuracy can accidentally create "mosaic cell lines" (also known as mosaicism) within the embryo, i.e., some of the cells are restored but others remain unrepaired. Mosaicism has been ascribed to the inability of CRISPR to correct all mutant genes after cell division occurred[10].

Still, researchers have pointed out that there are ways to overcome major targeting inefficiencies and difficulties posed by mosaicism in edited embryos. In a report dated August 2017, researchers have laid out how to achieve repair of a heterozygous four base pair deletion in MYBPC3, i.e., a genetic factor for hypertrophic cardiomyopathy. In their experiments, they repaired the genes of pre-implantation embryos, reaching 100% efficiency (n=58). This remarkable rate of efficiency was achieved through the co-injection of CRISPR/Cas9 with the mutant sperm during the ICSI at metaphase II wild-type oocytes stage, rather than at the more typical S-phase zygotic stage[11]. Timing played an essential role in this instance: the introduction of CRISPR/Cas9 before fertilization in fact triggered the editing and correction when only a single mutant copy of the targeted gene was present. The double-strand breaks (DSBs) brought about by Cas9 were mostly corrected by means of homology-directed repair (HDR), that is using the corresponding original maternal gene as template, rather than the injected oligonucleotide template. That fundamental change in the applied methodology made the correction possible for all embryos, thus solving almost entirely the issue of mosaicism. As a matter of fact, only one instance of mosaicism was observed, and it would have still been fit for transfer; its blastomeres were essentially and evenly wild type, but some of them had been corrected using the maternal allele as template and others through the oligonucleotide template that had been injected[12].

In order to make a thorough assessment of the off-target effects in human embryos, it is necessary to take into account whole genome sequencing (WGS) to measure the margin of error. Nonetheless, sometimes only the sites in the genome that are highly homologous to the targeted repair site are accounted for, as off-target effects may be enriched here. Overall, if this technology is ever to become a mainstream tool in clinical care, further evaluations of off-target effects must be undertaken in order to clear up some unresolved points. For instance, in light of the mosaicism issue, at what stage of embryonic development should an assessment be made? What

and how many areas should be tested to achieve an acceptable degree of clinical validation? What kind of testing protocols and platforms may be deemed adequate, in terms of depth and resolution, in order to assess and quantify off-target unwanted effects and rule out the possibility of allele dropout?[13] Such fundamental issues need answers, in order to make a realistic appraisal as to the future prospects of CRISPR/Cas9 and similar technological innovations with revolutionary implications.

Potential Impact and Future Applications and Developments

As already mentioned, the CRISPR/Cas9 system has a great margin of growth and potential, thanks to its capability to edit any cell, either human, animal or plant, in order to correct even the slightest mutations at the genetic level. In addition to its applications in basic research, CRISPR/Cas9 is usable in virtually all biotechnological settings[14].

According to the Hinxton Group, at least four basic areas may soon achieve significant results through the use of CRISPR techniques: (1) research on genome editing itself, particularly in terms of its further improvement and scope of application; (2) genome editing as a tool to tackle fundamental issues arising from human and animal biology; (3) research to achieve preliminary data for the development of human somatic applications; and lastly (4) research to inform the feasibility of developing safe human reproductive applications[15]. Still, due to the degree of inaccuracy still associated with CRISPR, its unwanted side-effects constitute according to some "great good and great harm"[16].

From a clinical standpoint, experimentation has been in development for appraising the opportunity to use CRISPR to treat HIV at the genomic level, hoping to achieve genetically altered CD4 T lymphocytes (CD4 cells) that help coordinate the immune response through the stimulation of immune cells; that could potentially replace current anti-viral therapies[17]. In addition, future CRISPR applications may include treatment for Duchenne muscular dystrophy (DMD), caused by dystrophin gene mutations. By reproducing such mutations in mice, researchers have been able to prove that excising a precise DNA strand which contains the DMD-causing mutation, the mice exhibited noticeable improvements and higher degrees of muscular functionality, including respiratory and cardiac muscles[18,19].

In addition, nowadays CRISPR constitutes an experimental tool in cancer research[20]: CRISPR techniques can modify at precise DNA locations. Scientists have attempted to identify and establish the role of some genomic mutations in carcinogenesis and evaluate the impact of the genes that can be knocked out through CRISPR on the survival and the replication of cancerous cells. A noteworthy instance of that approach is the CAR-T cells therapy, administered to patients with severe forms of immunodeficiency, genetic diseases, and blood cancers[21]. The CRISPR/Cas system editing works well also for non-coding RNA (ncRNA; microRNA, long noncoding RNA, circular RNA, etc.)[22] which have well established roles in several human cancers, such as those of the urogenital system[23-25]. For example, this approach has been successfully used to target UCA1 in bladder cancer[26]. Similarly, the use of this technique in the treatment of endometrial cancers is established for coding genes[27]; hopefully, an approach similar to that for UCA1 is also conceivable for ncRNA characterizing gynecological tumours. Moreover, germline editing can be greatly effective in directly altering and correcting fundamental genetic mutations and related diseases, allowing for the development of tissue-based treatments for various conditions, by knocking out disease-causing genes, thus correcting such harmful mutations or even by inserting new genes with protective effects. Hence, new targets can be identified, and more effective drugs developed accordingly[28].

Promising results[29,30] have been achieved in the treatment of diseases such as spinal muscular atrophy (Sma1). New genome-editing techniques were developed in China in 2018, aimed at fixing the mutation responsible for Marfan Syndrome; the procedure was first experimented on *in vitro* stem cells, and later on developing human embryos[31]. The technique ultimately proved acceptably safe, with no off-target instances detected. It is arguably an important result, in that it can be viewed as the first step towards curing a potentially lethal genetic condition severely affecting the cardiovascular system, particularly the mitral and aortic valves[32].

Detection and Treatment of Viral Diseases: The Examples of SARS-CoV-2 and HIV

In 2018, Myhrvold and collaborators demonstrated that it is possible to detect Zika virus (ZIKV) and dengue virus (DENV) in patient

samples at concentrations as low as 1 copy per microliter, using the SHERLOCK platform (see below)[33]. Since then, several CRISPR-based approaches have been developed for virus detection, with the promise to be at least as sensitive as those PCR-based, despite the scientific history of the former technology is only ca. 2 years old compared to the >20 years of PCR-based industry tests. Consequently, also different Cas proteins are used, such as Cas9 and Cas12 (both cutting DNA) or Cas13 (cutting RNA), depending on the protocol to be used and the aim of the kit (diagnosis or treatment). In particular, we recall here two different approaches, that are to date the most promising in COVID-19 diagnosis. The first approach, already cited above, is called SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) that is based on Cas13a. This platform combines nucleic acid pre-amplification with CRISPR/Cas enzymology for specific recognition of desired DNA or RNA sequences and a detection method based on fluorescence and colorimetric readouts that provide results in <1 h with a setup time of less than 15 min[34]. The second approach is DETECTR (SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter)[35], that uses Cas12. This assay performs simultaneous reverse transcription and isothermal amplification using loop-mediated isothermal amplification (RT-LAMP).

In this system, the cleavage of a reporter molecule induced by the Cas protein causes the release of a detectable signal. The fundamental limitation of these techniques in the treatment of the disease, beyond its diagnosis, is due to the specificity of Cas9 and Cas12 for cutting DNA, while the viral genome is an RNA. For this, a treatment protocol based on Cas13, which specifically targets RNA, is envisable[36]. In this perspective, a platform for this purpose is under extensive experimentation, although to date, no CRISPR-based therapy has been approved for human use. This platform is called PAC-MAN (Prophylactic Antiviral CRISPR in huMAN cells) and it is based on Cas13d[37]. In this case, the CRISPR/Cas13 complex directly cleaves the viral genome, inactivating it; it has been shown to efficiently work *in vitro* on human lung epithelial cells and to promote the degradation of both SARS-CoV-2and influenza A virus (IAV)[38]. In particular, for SARS-CoV-2, best results had been obtained by targeting two highly conserved regions, one containing the RNA-dependent RNA polymerase (RdRP) gene, which is pivotal for its proliferation, and the other the Nucleocapsid (N) gene, which encodes the capsid protein for viral packaging. Notably, if the expected results are achieved, CRISPR-based tools for diagnosis have several advantages over PCR: 1) they are much faster (less than one hour vs. 24 hours)[35,38,39]; 2) require low infrastructure thanks to the isothermal amplification running at room temperature, i.e., no requirement for a thermocycler; 3) use neither expensive nor cold-stored reagents; 4) possibly, they have a better sensitivity (best results in vitro for these systems outperform average standard qRT-PCR sensitivity by 6x)[35]. A few weeks ago, a new test was announced to test for SARS-CoV-2, based on Cas13a, to further lower the reaction time down to just 5 minutes, by eliminating the amplification phase[40]. Although its sensitivity is not yet very high (100 virus/microliter) compared to PCR, this approach still yields a quantitative result, which could be very valuable to estimate the viral load of the patients, a variable that in some instances may be crucial for the subsequent outcome[41]. That contribution may prove beneficial, particularly in times of pandemic and overwhelmed hospitals, with all the clinical and ethical implications that scenario entails[42].

Attempts to use the CRISPR/Cas system to treat viral diseases had great resonance in both the scientific community and media, when some groups tried to make human embryos resistant to HIV infection. The approach aimed to directly modify the cells of a preimplantation embryo, with all ensuing ethical issues already discussed in this manuscript. The story of the Chinese biophysicist He Jiankui (and his staff members, Zhang Renli e Qin Jinzhou) is well known, as well as its ending: the scientist was fired by his University in January 2019 and, one year ago, he was sentenced to three years in prison, to pay a fine, and banned from continuing research in the field and applying for research funding[43]. A more ethical approach was instead used by another Chinese group, who used treated stem cells in a patient with HIV and acute lymphocytic leukemia[44]. The idea was to knock down the CCR5 gene because CCR5-null blood cells are largely resistant to HIV-1 entry, and at the same time also trying to treat the running leukemia with the transplant of the stem cells. Unfortunately, despite the complete remission of leukemia and the donor cells carrying the ablated CCR5 persisted for more than 19 months without gene editing-related adverse events, on-

ly 5% of CCR5 disruption in lymphocytes was detected, indicating the need of further technique improvement.

A possible evolution, and hopefully a more efficient development, of this approach is currently under investigation, using the CRISPR/Cpf1 (also known as CRISPR/Cas12a) system[45].

CRISPR Gene Editing in Human Embryos: Prospects and Concerns

Genetic editing of human embryos has been carried out by using supernumerary embryos and has largely failed to produce the results hoped for by researchers. CRISPR/Cas9 has been applied in an attempt to knock out the gene responsible for beta thalassemia. Out of 86 embryos used in the trial, just 4 ultimately acquired the desired change at the genetic level, whereas off-target editing occurred in all the others[46]. In a second trial, a group of Chinese scientists had carried out the same intervention on 26 human embryos not meant for implantation, in order to knock out the CCR5 gene, which would have made those embryos impervious to the HIV virus[47]. In that instance, however, the intended genetic alteration was achieved in just 4 out of 26 embryos used overall, with off-target unpredictable and potentially harmful alterations in the other 22 embryos. That initiative has drawn criticism and ignited heated debate, which caused the two prominent scientific journals "Nature" and "Science" to refuse publication of the study.

While CRISPR/Cas9 undoubtedly has amazing therapeutic potential, it is just as undeniable that its introduction to clinical practice is bound to engender ethical and social concerns.

Such techniques may in fact be used to bring about heritable genetic traits, lead to "designer babies", in what could become a modern version of eugenics (i.e., the biological enhancement and selection of the human race), possibly resulting in dangerous, uncontrollable modifications, and even upset entire ecosystems. Genome editing poses serious concerns not only related to human applications, but to virtually all organisms and environments. CRISPR applications for pest control, for instance, may trigger unwanted effects and mutations, brought about by gene drive interventions, which might even lead to the extinction of entire species (or the accidental spread of others), affecting ecosystems in a major way[48]. Besides, what genetic changes could CRISPR produce if applied on human germline? The newly found ability to genetically alter human embryos has ignited great controversy and brought to light opposing views in the scientific and academic communities as to the ethical standards and governance principles that should bind human genome editing[49].

Opposing lines of philosophical analysis have developed in reaction to studies centred around genome editing at the embryonic level prior to implantation and aimed at human bioenhancement and heritable genetic alterations. Indisputably, the scientific ability to edit the human germline poses serious doubts and quandaries concerning the blurring of the lines between therapy and eugenics, the notion of human dignity, and ultimately, the very role that science should play at the beginning of life stage. The most vexing issues and worries in that regard may be summarized in one question: when genome editing techniques become mainstream, will scientists merely replace defective genes with healthy ones, or will they actively work towards "improving" or enhancing the genetic profile by altering traits such as, for instance, physical endurance, strength, cognitive capabilities, or somatic features?

Among all the controversies sparked by genome editing techniques, the one on the human applications of biotechnologies is likely the most divisive. CRISPR applications on adult somatic cells is not controversial, since scientists merely use such techniques to treat a congenital condition. The patients who take part in the experimentation, in compliance with all the regulations governing pharmaceutical and research trials, are adults suffering from major genetic conditions, various forms of cancers, HIV-AIDS, muscular dystrophy and the like. Patients with dystrophy, for example, have a dystrophin deficiency caused by deletions of one or several exons of the dystrophin DMD gene. CRISPR may be used to correct that mutation, thus enabling the patient's muscles to produce dystrophin, at least to some extent, so as to recover some degree of muscular mobility. It is worth stressing that such mutations are not heritable, hence not passed on to the patient's offspring. That is the main difference with Germline Genome Editing. Through *in vitro* fertilization and CRISPR genome editing, scientists can affect the development of embryos at the very early stages, by redesigning the DNA of humans yet to be born, thus altering the whole organism with heritable modifications[50].

The somatic and germline cell lines are not entirely distinct after all: they overlap in some cases.

Alterations in germline or embryonic cells may not be found in all the cells of the patient that underwent the editing procedure, hence they may be non-heritable. Furthermore, gene editing carried out at certain stages of development, particularly for fetuses in the womb, can give rise to a range of somatic and germline changes[51]; such interventions may lead to the birth of genetically enhanced individuals with heritable genetic traits. Only by testing and observing the newborn, can the success of such techniques be positively verified. It is safe to assume that the long-term consequences of such DNA-altering techniques are hardly foreseeable. Still, it can be concluded that the beginning of life stage may well become open to manipulation and potentially subjected to arbitrary individual choices[52].

Therapy vs. Enhancement: A Somewhat Ill-Defined Distinction

The issue of where to draw the line between therapeutic interventions and acts aimed at enhancing human capabilities at the genetic level is extremely complex and multifaceted. Genetic enhancement is part of the human enhancement framework[53].

The phrase "genetic enhancement" has been summarized as the use of highly sophisticated biotechnologies for adding genes to an individual genome, or altering the so-called non-disease genes, i.e., genes already included in one's genetic profile which do not cause genetic disorders or predispose one to develop diseases later on. Such treatments aim to enhance physical, cognitive or moral capabilities in the human being[54].

When scientists carry out CRISPR procedures aimed at alleviating the burden of disease[55] or disability, or even curing them, that intervention can be deemed therapeutic in nature, hence appropriate; on other hand, improving human capabilities and characteristics goes beyond merely treating and curing disease, and therefore constitutes an enhancement. Enhancement is aimed at a better, more efficient body (or mind), not a merely healthier one, which many ethicists deem ethically objectionable[56].

The notion of distinguishing therapy from enhancement appears reasonable, albeit quite ill-defined. Various interventions may in fact be deemed to fall within both categories: it is often challenging to identify the boundaries separating the two, since medical acts may be aimed at treating and improving certain conditions. Stimulants, for instance, may be used to treat attention deficit hyperactivity disorder (ADHD), but also as cognitive enhancers to aid intellectual efforts[57]; steroids help with inflammation, but are often used illicitly by athletes to gain a competitive edge. That rationale may be further clarified by taking a disabling condition such as dystrophy to draw an example: while it is ethically desirable to correct muscular atrophy at the genetic level in patients with that condition, the same cannot be said about a similar intervention aimed at improving physical performances in an athlete, albeit consenting. Vaccinations are another example of medical interventions to create immunity to certain diseases. Vaccination in itself, however, is a form of "enhancement" as well, because its benefits would not be naturally available to everyone. If it serves the purpose of preventing disease, on the other hand, vaccinations are forms of therapeutic preventive treatment. Scientists still wonder where the line should be drawn between treating patients and creating an unlikely "superhuman"[58].

How Far Can Scientists Push the Boundaries?

The fact that genome-editing techniques will likely become ever more widespread, and perhaps even mainstream, does not mean that they should be sanctioned and be deemed advisable. It is apparent, as admitted by numerous scientists, that for the time being, genome editing techniques are nowhere near safe enough, as proven by the research trials which have been herein expounded upon, and many more; it should be adamantly banned in all its applications except for non-reproductive purposes[59].

The unwanted effects that such procedure can bring about may not be immediately recognizable and are also quite unpredictable in terms of when they could manifest themselves in life. Extreme caution is therefore called for when dealing with such techniques. That extremely cautious approach, however, does not mean failing to take full advantage of all the opportunities offered by science for the common good of all humanity. Any act or attempt entails risks, which are impossible to eliminate, because that would mean wasting the opportunity to reap the benefits altogether. There is a considerable difference between waiting for genome editing techniques to become safer and more effective and entirely ruling out such avenues of research, lest the long-term, unpredictable risks be unreasonable.

In our opinion, the former option is far more sensible, since demanding the total absence of risks would be an unreasonable standard, never applied before in medical history. Let us take assisted reproductive technologies and *in vitro* fertilization: after decades of using such techniques, science still has no certainty as to the safety of such interventions; nonetheless, thousands of such procedures are carried out all over the world, with no significant rates of adverse outcomes. We believe that it is therefore necessary to abide by the rules and assessments of scientists, ethics committees and oversight commissions. The following principle should be viewed as a beacon light to guide our pursuits: research carried out on humans should never entail unreasonably high risks[60]. Inevitable risks should be minimized and weighed against possible benefits[61], while striving to meet all the scientific standards. When determining whether a risk is reasonably worth taking, it is essential not only to account for the likelihood of achieving a benefit, but also to assess the scope of said benefit. Hence, the larger the benefit, the greater the risk worth taking to achieve it. A core principle ought to be applied: higher risks can be taken when the likelihood of success is high, but such risks also require greater confidence in their likely efficacy[62].

Conclusions

Germline genome editing undoubtedly holds great promise for the future of medical science: the natural evolution of current genetic therapies. In order to effectively tackle some of the issues herein analyzed, the principle of caution and accountability towards future generations ought to be espoused. We firmly believe it is advisable to widen the scope of research into such techniques and the opportunities that they may offer, bearing in mind that such a line of research needs to be conducted in full compliance with technical-scientific and ethical recommendations. That is true for any kind of biomedical technology capable of fundamentally impacting the values and beliefs that we as humans hold dear.

A parallel can be drawn with assisted reproductive technologies or end of life issues: both have brought about a sea-change in the way ethics, morals and new social needs are reconciled, and both have been regulated with varying degrees of restrictions, in Italy and elsewhere[63-65]. That is why a broad-ranging debate should be started on the subject, and the scientific community is already moving in that direction. Nonetheless, there is no discounting the doubts expressed by many scientists, among whom British biologist Ian Wilmut, the "father" of the genetically-modified sheep Dolly, as to the real possibility to achieve meaningful results from embryonic stem cells experimentation.

Such uncertainties have led some scientists to opt for adult stem cells. In that respect, it is worth mentioning the research studies by Japanese stem cell researcher Shinya Yamanaka[66], the 2012 Nobel Prize winner for medicine. He has undertaken and developed research on induced pluripotent stem cells, dubbed by some "ethical stem cells", since they do not entail the destruction of embryos, whether by using supernumerary embryos or by producing new ones for research purposes; conversely, such techniques are capable of reprogramming adult cells until they regress to a state close to that of embryos, hence without the need to use actual ones. Such option could be further developed as new innovative technologies such as 3D bioprinting progress and become available, with potentially immense benefits in regenerative medicine: the bioprinting of autologous iPSC-derived organs cancels the risk of immune rejection following organ transplants[67].

Conflict of Interest
The Authors declare that they have no conflict of interests.

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