

## GENE THERAPY: RECENT DEVELOPMENT IN THE TREATMENT OF VARIOUS DISEASES

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### ABSTRACT

Gene therapy (use of genes as medicines) is basically to correct defective genes responsible for genetic disorder by one of the following approaches. Gene therapy states and remains an experimental discipline and many researches remain to be performed before the treatment will realize its potential. The ideal design of a gene therapy strategy would first take into account the molecular basis of a disease process and then effectively tailor gene transfer techniques to mitigate toxicities and improve the effectiveness of existing therapies. A gene therapy strategy modelled after the ADA protocol could play a role in delivering neuronal growth factors to the nervous system of patients with neurodegenerative disease. Cells from various tissues could be removed from a patient, be grown in tissue culture where they could be stimulated to replicate, be genetically modified with retroviral vectors carrying a gene of therapeutic importance, and then be implanted into a brain with the intent to increase the local delivery of biologically active molecules. Genetic medicines are simple in concept, but challenging to make a therapeutic reality. We first outline the general concepts that are applicable to genetic medicines. Gene therapy has the potential to eliminate and prevent hereditary diseases such as cystic fibrosis and is a possible cure for heart disease, AIDS and cancer. A gene therapy strategy known as viral-directed enzyme prodrug therapy has used retroviral vectors to eliminate some types of experimental brain tumors in rodent model. Finally, from an ethics standpoint, it is important to consider whether medicine should surrender to the rule of technology or commit to a more responsible steering of the course of progress.

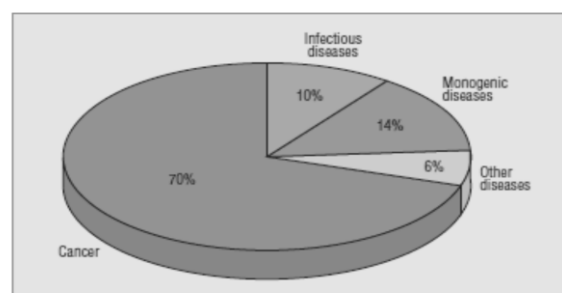
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### INTRODUCTION

Genes, the functional unit of heredity, are specific sequences bases that encode instructions to make proteins. Although genes get a lot of attentions, it is the proteins that perform most life functions. When genes are altered, encoded proteins are unable to carry out their normal functions, resulting in genetic disorders. Gene therapy (use of genes as medicines) is basically to correct defective genes responsible for genetic disorder by one of the following approaches.<sup>1,2</sup>

Gene therapy states and remains an experimental discipline and many researches remain to be performed before the treatment will realize its potential. Majority of the gene therapy trials are being conducted in United States and Europe, with only a modest number in other countries including Australia. Scope of

this approach is broad with potential in treatment of diseases caused by single gene recessive disorders (like cystic fibrosis, hemophilia, muscular dystrophy, sickle cell anemia etc), acquired genetic diseases such as cancer and certain viral infections like AIDS.<sup>3,4</sup>



Proportion of protocols for human gene therapy trials relating to various types of disease

Fig. 1:5

Three decades ago, scientists developed techniques that enabled them to manipulate the genome of bacteria. Soon afterwards, advances permitted researchers to clone and then transfer genes into animal cells. It was during that time that progress in research laboratories fuelled speculation that gene transfer might one day be used to treat disease in human patients. Gene transfer techniques have now established a foothold in modern clinical medicine.<sup>6-13</sup>

### Gene Therapy for Inherited Disease

The ideal design of a gene therapy strategy would first take into account the molecular basis of a disease process and then effectively tailor gene transfer techniques to mitigate toxicities and improve the effectiveness of existing therapies. A successful example of this strategy is provided by the efforts of investigators at the National Institutes of Health who developed a somatic cell gene therapy approach for the treatment of the severe combined immune deficiency syndrome resulting from adenosine deaminase (ADA) deficiency.<sup>9,14-18</sup>

Patients afflicted with ADA deficiency can be cured by matched bone marrow transplants providing immune cells with normal ADA levels. On that basis, it was proposed that the transfer of the human ADA gene into T lymphocytes from ADA-deficient patients might have the same effect.<sup>4</sup> An investigational approach was then designed in which mononuclear cells were isolated from the blood of patients with ADA deficiency, stimulated to proliferate in tissue

culture, infected with a retroviral vector carrying the human ADA gene, and then returned to the patient.<sup>15-17</sup> A pilot case in which nerve growth factor was infused into the lateral ventricle in a patient with Alzheimer's disease showed results that included increased cortical blood flow, normalization of electroencephalographic abnormalities, and improvement in some psychological tests.<sup>19</sup> A similar approach has been shown to be effective in reducing abnormal neurologic activity resulting from the destruction of nigrostriatal pathways in rodent models of Parkinson's disease after transplantation of cells modified to produce levodopa.<sup>20</sup>

Although these therapies do not address the mechanisms that initiate neurodegenerative disorders, they provide experimental evidence that the delivery of trophic factors and substrates by genetically modified somatic cells can alter disease progression and open new avenues for further clinical investigation. A gene therapy strategy modeled after the ADA protocol could play a role in delivering neuronal growth factors to the nervous system of patients with neurodegenerative disease. Cells from various tissues could be removed from a patient, be grown in tissue culture where they could be stimulated to replicate, be genetically modified with retroviral vectors carrying a gene of therapeutic importance, and then be implanted into a brain with the intent to increase the local delivery of biologically active molecules.<sup>21,22</sup>

**Table 1: Summary of approved and published current clinical gene therapy protocols<sup>23</sup>**

Disorder	Objective	Target cells	Mode of delivery	Countries with protocols
ADA deficiency	ADA replacement	Blood	Retrovirus	Italy, the Netherlands, United States
Alpha-1-antitrypsin deficiency	Alpha-1-antitrypsin replacement	Respiratory epithelium	Liposome	United States
AIDS	Antigen presentation HIV inactivation	Blood, marrow Blood, marrow	Retrovirus	United States
Cancer	Immune function Enhancement, Tumour ablation, Tumour ablation	Blood, marrow, tumour	Retrovirus	Austria, China, France, Germany, Italy, the Netherlands, United States
Cystic fibrosis	Cystic fibrosis transmembrane regulatory enzyme replacement	Respiratory epithelium	Adenovirus, liposome	United Kingdom, United States
Familial hypercholesterolemia	Replacement of low-density lipoprotein receptors	Liver	Retrovirus	United States
Fanconi's anemia	Complement group C gene delivery	Blood, marrow Blood, marrow	Retrovirus	United States
Gaucher's disease	Glucocerebrosidase replacement	Blood, marrow Blood, marrow	Retrovirus	United States
Hemophilia B	Factor IX replacement	Skin fibroblasts	Retrovirus	China
Rheumatoid arthritis	Cytokine delivery	Synovium	Retrovirus	United States

## Process of Gene Therapy

There are 2 types of gene therapy

### 1. Germ line gene therapy

Where germ cells (sperm or egg) are modified by the introduction of functional genes, which are integrated into their genome. Therefore changes due to therapy would be heritable and would be passed on to later generation. Theoretically, this approach should be highly effective in counteracting genetic disease and here dietary disorders. But at present many jurisdictions, a variety of technical difficulties and ethical reasons make it unlikely that germ line therapy would be tried in human beings in near future.<sup>24</sup>

### 2. Somatic gene therapy

Where therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only and will not be inherited by the patients offspring or any later generation.<sup>25</sup>

## Approaches of gene therapy

### 1. Gene modification

- A. Replacement therapy
- B. Corrective Gene therapy

### 2. Gene transfer

- A. Physical
- B. Chemical
- C. Biological

### 3. Gene transfer in specific cell line

- A. Somatic gene therapy
- B. Germ line gene therapy

### 4. Eugenic approach (gene insertion)

Other forms of genetic engineering include gene targeting and knocking out specific genes via engineered nucleases such as zinc finger nucleases, engineered I-CreI homing endonucleases, or nucleases generated from TAL effectors. This approach is currently being used in several human clinical trials.<sup>26</sup>

## Genetic medicines: treatment strategies for hereditary disorders

Genetic medicines are simple in concept, but challenging to make a therapeutic reality. We first outline the general concepts that are applicable to genetic medicines. We then review the genetic medicine strategies being developed to treat monogenic disorders, including those that involve the use of SSCs (excluding combined SSC-gene-transfer strategies, which are discussed in the section on gene transfer), gene transfer, RNA modification, and ESCs. For each of these strategies we describe the current status of applying these therapies to treat here dietary human disorders and the biological challenges in making genetic medicine therapies a reality.<sup>27</sup>

## Genetic medicines — general considerations Global challenges

The main biological barriers for all genetic medicines are the delivery and maintenance of new genetic information. For gene transfer therapy, this requires circumventing immune defences that are raised against the vectors that carry the new gene, transferring the gene to sufficient numbers of cells to modify the phenotype, and controlling the expression of the gene.<sup>27,28</sup>

## Somatic stem cell therapy

In a sense, organ transplantation for a monogenic hereditary disorder is the ultimate 'genetic medicine', in that it involves replacing, along with the relevant SSCs and differentiated cells, the organ that is malfunctioning secondary to the abnormal phenotype. Organ transplantation for hereditary disorders has included replacing the liver, kidney, lung and hear.<sup>29</sup>

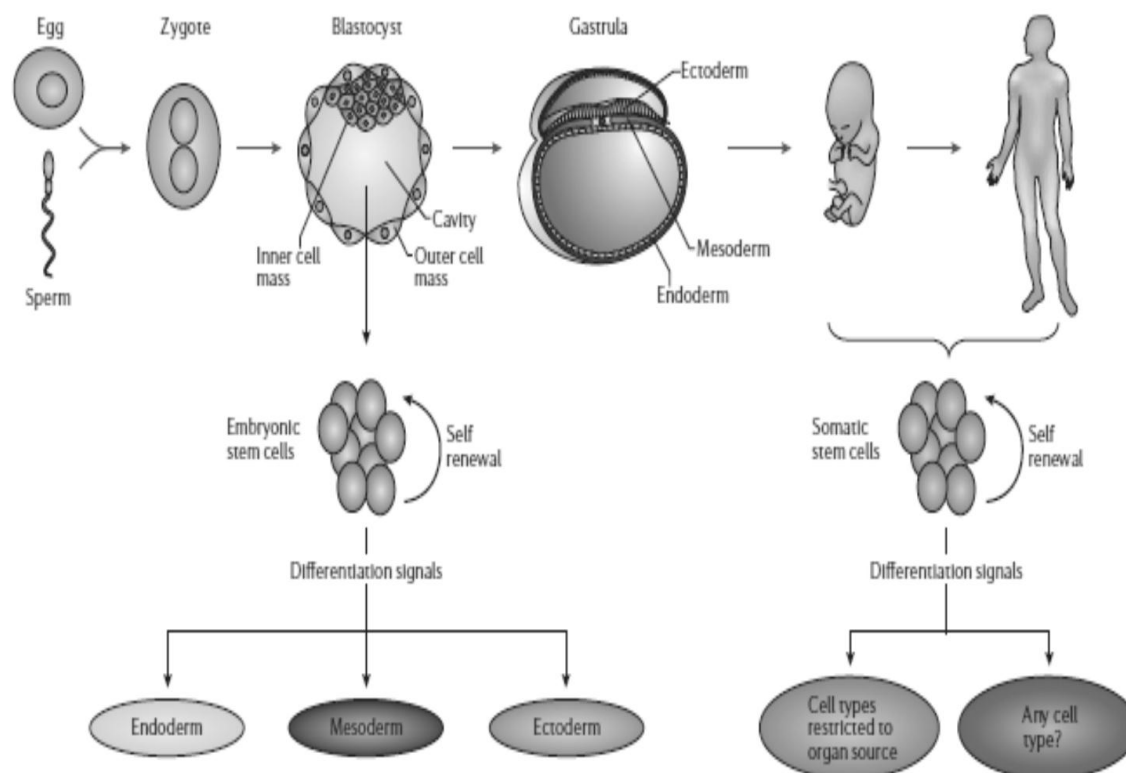


Figure 1 | **Embryonic and somatic stem cells as a source of genetic medicines.** The fusion of sperm and egg gametes during human fertilization establishes a diploid zygote and initiates a series of cell divisions that result in a multicellular embryo. The blastocyst stage is characterized by the presence of a blastocyst cavity, outer cell mass and inner cell mass. Embryonic stem cells are derived from the inner cell mass of the blastocyst. Embryonic stem cells in culture are capable of self-renewal without differentiation and are able to differentiate into all cell types of the endoderm, mesoderm and ectoderm lineages using appropriate signals. *In utero*, the blastocyst implants and all three embryonic germ layers are formed during gastrulation. Somatic stem cells are present in many fetal and post-natal tissues. Somatic stem cells are also capable of self-renewal and, with appropriate signals, differentiate into various cell types from the organ from which they are derived. The extent to which they are capable of differentiating into cell types from alternative lineages is controversial.

Fig. 2:2

### Vectors in gene therapy

Some of the different types of viruses used as gene therapy vectors

#### Retroviruses

A class of viruses that can create double-stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosomes of host cells. Human immunodeficiency virus (HIV) is a retrovirus. eg:- One of the problems of gene therapy using retroviruses is that the integrase enzyme can insert the genetic material of the virus into any arbitrary position in the genome of the host; it randomly inserts the genetic material into a chromosome. If genetic material happens to be inserted in the middle of one of the original genes of the host cell, this gene will be disrupted

(insertional mutagenesis). If the gene happens to be one regulating cell division, uncontrolled cell division (i.e., cancer) can occur. This problem has recently begun to be addressed by utilizing zinc finger nucleases.<sup>30</sup>

#### Adenovirus

To avoid problem of inserting genes at wrong sites, some researchers have turned to other types of viruses. A class of virus with double stranded DNA genome that can cause respiratory, intestinal and eye infection (especially the common cold). When these viruses infect a host cell, they introduce their DNA molecule into the host. The genetic material of the adenovirus is not incorporated into the host cell's genetic material. The DNA molecule is left free in the nucleus of the host

cell, and the instructions in this extra DNA molecule are transcribed just like any other gene (Figure 4). Adenovirus also can infect a broader a broader variety of cells than retrovirus, including cells that divide more slowly, such as lungs cells. However, adenovirus also are more likely to be attacked by the patient's immune system, and the high levels of virus required for treatment often provoke an undesirable inflammatory response. Despite these drawbacks, this vector system has been promoted for treating cancer of liver and ovaries and indeed the first gene therapy product to be licensed to treat head and neck cancer is Gendicine, p53 based adenoviral product. 17 Concern about the safety of the above vectors was raised after the 1999 death of Jesse Gelsinger while participating in a gene therapy trial.18 Since then, work using adenovirus vector has focused on genetically crippled version of the virus<sup>35</sup>.

#### A. Cis and trans-acting elements

Replication-defective vectors always contain a "transfer construct". The transfer construct carries the gene to be transduced or "transgene". The transfer construct also carries the sequences which are necessary for the general functioning of the viral genome: packaging sequence, repeats for replication and, when needed, priming of reverse transcription. These are denominated cis-acting elements, because they need to be on the same piece of DNA as the viral genome and the gene of interest.<sup>31</sup>

#### B. Hybrid methods

Due to every method of gene transfer having shortcomings, there have been some hybrid methods developed that combine two or more techniques<sup>32</sup>. virosomes are one example; they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods<sup>33</sup>.

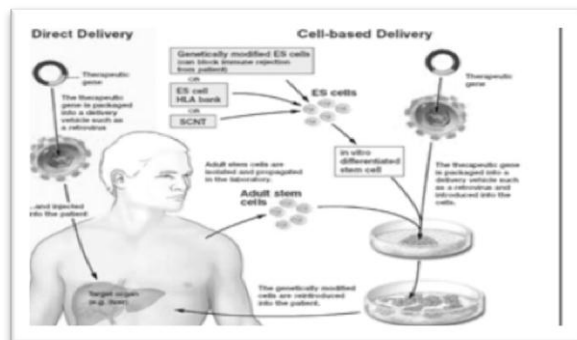


Fig. 3:<sup>34</sup>

### Advantages and disadvantages of gene therapy

#### Advantages of gene therapy

- A. In case of 'silence' a gene. In the case of someone with HIV, which had not yet developed into AIDS, scientists could save them the pain and suffering of the disease by using gene therapy to 'silence' the disease before its onset.
- B. Gene therapy has the potential to eliminate and prevent hereditary diseases such as cystic fibrosis and is a possible cure for heart disease, AIDS and cancer.
- C. These sceptics would almost certainly choose gene therapy, especially if it was the last hope for them or one of their loved ones – as is the case for many gene therapy patients.<sup>36</sup>

#### Disadvantages of Gene Therapy

##### A. Short-lived nature of gene therapy

**B. Immune response** Any time a foreign object is introduced into human tissues, the immune system has evolved to attack the invader. The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a possibility. Furthermore, the immune system's enhanced response to invaders makes it difficult for gene therapy to be repeated in patient.<sup>37</sup>

**C. Multi gene disorders** Conditions or disorders that arise from mutation in a single gene are best candidates for gene therapy. Unfortunately, some of the most commonly occurring disorders, such as heart disease, high blood pressure, Alzheimer's disease, arthritis and diabetes, are caused by the combined effects of variations in many genes. Multigenic or multifactorial disorders would be especially difficult to treat effectively using gene therapy.<sup>38</sup>

### Gene therapy process

#### 1. Haematopoietic-stem-cell gene therapy

HSCs have long been a preferred target for *ex vivo* gene therapy.<sup>39</sup> HSC gene therapies that incorporate these new vectors have been tested in severe inherited diseases of the immune system (Wiskott-Aldrich syndrome (WAS) and X-linked severe combined immunodeficiency (SCID-X1))<sup>40-42</sup>. In children with SCID-X1, the development and function of the immune system is impaired owing to deficiencies in the receptors for certain cytokines that are essential for immune-cell development, whereas those with WAS have deficiencies in a cytoskeletal adaptor that is required for assembling the immunological synapse.<sup>43</sup>

HSC gene therapy is administered by *ex vivo* gene transfer into haematopoietic progenitors. First, the cells are purified using the CD34

surface marker from other leukocytes harvested from the bone marrow or mobilized peripheral blood of the recipient. Next, they are cultured for 2–4 days in the presence of growth-stimulating cytokines while being exposed to vectors carrying an expression cassette for the corrective transgene. Before the modified cells can be administered, the recipient is treated with a preconditioning chemotherapy regimen. This depletes endogenous progenitors and differentiated cells in the bone marrow — as well as the lymphoid organs, in some cases — and favours engraftment of the *ex vivo* gene-corrected cells. Preconditioning results in considerable early morbidity owing to transitory blood-cell depletion, immunodeficiency and mucosal damage, which place the recipient at risk of severe infection. It also causes delayed morbidity owing to the risk of developing chemotherapy-induced secondary tumours and infertility.<sup>44</sup>

## 2. Liver-directed gene therapy

The liver has long been a preferred target for *in vivo* gene therapy.<sup>45</sup> This major internal organ and central metabolic hub receives an abundant blood supply through an extensive bed of sinusoids with highly permeable walls — a structure that facilitates the access of blood-borne particles, such as viruses, to hepatocytes. Hepatocytes are long-lived and robust protein factories that can efficiently release their products into the blood circulation. Stable transgene delivery to the liver could therefore provide a strategy for treating several inherited metabolic diseases and plasma-protein deficiencies, notably those of coagulation factors. Major hurdles to liver-directed gene therapy include the potential toxicity of an acute inflammatory response to the bolus administration of viral particles into the bloodstream, and the inactivation of these particles by pre-existing virus-specific antibodies and clearance by phagocytes that line the sinusoidal walls of the liver and spleen. If humoral or cellular immunity is triggered against the transgene product, the therapeutic activity of gene therapy could be inhibited in the circulation or the modified cells might be eliminated by cytotoxic T cells, respectively.<sup>46-47</sup> Liver-directed gene therapy has been tested mainly in the treatment of severe haemophilia B using vectors derived from the human parvovirus, adeno-associated virus (AAV).<sup>48</sup>

### T-cell immunotherapy for cancer

T cells are also popular targets for *ex vivo* gene therapy. Such therapies aim mostly at boosting the adaptive immune response against cancer

and chronic infections such as HIV.<sup>49,50</sup> Autologous T cells can be harvested readily from the peripheral blood and expanded *ex vivo*. Cells are then transduced with a  $\gamma$ -RV or lentiviral vector expressing an exogenous T-cell antigen receptor (TCR) that is specific to a cancer-associated antigen or an antiviral molecule, and infused back into the patient. The use of T cells for cancer immunotherapy arose from seminal observations of objective within a certain type of tumour and that cannot be lost, even to evade immune clearance. Recent studies, however, indicate that most spontaneous or elicited tumour-specific immune reactivity is instead directed against ‘passenger’ neoantigens, which uniquely originate from random mutations that accumulate within individual tumours.<sup>51-54</sup>

### Other relevant developments

There are several other advances in the gene-therapy field that could not be discussed in detail in this Review. These include applications in neurodegenerative diseases that have reached the clinical-testing stage. For example, good safety but limited efficacy has been demonstrated for the delivery of transgenes to the brain by AAV or lentiviral vectors.<sup>55-57</sup>

Adenoviral vectors of simian origin are also being assessed for their ability to induce humoral and cellular immunity through vaccination: encouraging results have already been seen in emerging or widespread infectious diseases that have long resisted conventional attempts.<sup>58,59</sup>

### Ethical and Social Consideration

Gene therapy is a powerful new technology that might have unforeseen risks, scientists first develop a proposed experiments i.e. protocol, that incorporates strict guidelines. After the approval from FDA, the organization continues to monitor the experiment. In the course of a clinical trial, researchers are required to report any harmful side effects. Critics and proponents all agree that risks of gene therapy must not be substantially larger than the potential benefit. Gene therapy poses ethical considerations for people to consider.<sup>60</sup>

### Application of Gene Therapybrain Tumors

The challenges that must be addressed in the design of gene therapy protocols for nervous system disease include the lack of knowledge about the genetic changes that lead to disease, the complexity of the structure and function of the nervous system, the limitations of effective techniques to deliver genes to the nervous system, and the possible toxicities of available vectors. For several reasons, these limitations

have had less effect on the development of gene therapy strategies to treat tumors of the nervous system. First, most brain tumors are localized masses of proliferating cells surrounded by normal, postmitotic brain tissue; therefore, retroviral vector-mediated gene transfer can be selective for replicating tumor cells and can spare normal brain. Second, insertional mutagenesis is less of a concern when neoplastic cells are the target for gene transfer. A gene therapy strategy known as viral-directed enzyme prodrug therapy has used retroviral vectors to eliminate some types of experimental brain tumors in rodent models.<sup>61-67</sup> The basis of this approach is straightforward: a gene encoding an enzyme that converts an inactive prodrug into a therapeutically active metabolite is transferred selectively into tumor cells. This enzyme can then mediate a cytotoxic effect in the infected cell. A key feature of this therapy is that retroviral vector mediated gene transfer requires dividing cells.<sup>68-70</sup>

#### THE FUTURE OUTLOOK

Gene therapy could be poised to become an important new approach for the third millennium because its reach extends well beyond that of conventional drugs. Gene therapy enables the targeted delivery of information-rich gene-based cassettes that facilitate the stable, sustained and regulated expression of biological agents. Furthermore, when combined with cell therapy, it turns cells into smart vehicles for targeted gene delivery. As exemplified in the studies discussed in this Review, gene therapy directs powerful biological processes towards the goals of disease correction, tissue repair and regeneration. For instance, the stability, fidelity and amplification of the delivered therapeutic can be guaranteed by transferring information by genetic mechanisms. The homing and trafficking mechanisms of cells in the human body can be used to target gene-based therapeutics to specific tissues and disease sites. Gene therapy also makes use of the regenerative potential of stem cells and transplantation as well as the biological weapon of immunity, which is exploited for the specific elimination of transformed or infected cells. By taking advantage of these inbuilt biological capabilities, gene therapy has the potential to address the substantial unmet medical needs of both rare and common severe diseases, which will benefit both patients and — more broadly — society. Major challenges must still be addressed before this promise can be realized. For example, the efficacy and safety of gene-transfer vectors should be improved by further engineering their

design and composition, which could include combining the biological features of different viruses with synthetic molecules. These advances will enable vectors to target tissues and cell types precisely.<sup>71-73</sup>

Finally, from an ethics standpoint, it is important to consider whether medicine should surrender to the rule of technology or commit to a more responsible steering of the course of progress. For instance, the avenues that are being opened to intervention by emerging technologies could undermine our self-perception and self-determination as we end up viewing ourselves as the evolutionary product of DNA that has become self-conscious and can edit itself to shape its progeny as and when it desires. The call for a moratorium on applying genome editing to human germline cells highlights forthcoming ethical dilemmas.<sup>74-75</sup>

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