The Emergence of a Novel Anti-Neoplastic Era in Gene Therapy for Cancer: A Review Study

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Abstract:
Cancer is still one of the leading causes of death in the world. The chance of getting the disease is linked to genetic and epigenetic factors. When it comes to treating local cancer, radiotherapy and surgery work the best. Chemotherapy, hormonal, and biological therapies can reach the rest of the organs and tissues through the circulatory system when the tumour has spread. Despite traditional treatments, patients' prognosis has not improved significantly. The toxicity and nonspecific destruction of normal cells required the need to develop targeted and efficient alternative treatments, with gene therapy being one of the most promising techniques. Recent advances in gene therapy have improved the survival rate and life expectancy of patients, as well as treatment possibilities. The most advanced and currently used techniques are CAR-T cell therapy, oncolytic virotherapy and the CRISPR gene editing technique. This work details the advances in efficacy in gene therapy after carrying out several clinical trials, as well as the obstacles and possible future solutions of the main techniques currently used for cancer. Gene therapy is an alternative to antineoplastics that, while not yet a cure for this illness, shows promise and may eventually be included to the standard therapeutic toolkit. Gene therapy is a promising antineoplastic approach that, while not a cure, holds great potential for future medical advancements.

Keywords: Anti-Neoplastic Era, CAR-T therapy, gene therapy, CRISPR cancer.

Introduction
Cancer is more than a single disease; it is defined as a group of related diseases. Normally, the cells in our body divide to form new cells, die when they are damaged or degraded, and are replaced by new cells (Brown et al., 2023). It is an orderly process. Cancer involves uncontrolled cell division and resistance to cell death. A tumour is an abnormal growth made up of cancer cells. Haematological cancers, on the other hand, have cells that grow and spread through the lymphatic, blood, and bone marrow systems. Its characteristics include continuous proliferative signaling, resistance to cell death, replicative immortality, cellular energy deregulation, angiogenesis, invasion and metastasis, and evasion of immune destruction (Tong et al., 2022).

Malignant means that the tumour has cancer cells in it. This kind of cancer can spread to
nearby tissues or even to other parts of the body through the lymphatic or circulatory systems, creating new tumours far away from the original one. This is known as metastasis. Cancer that has spread has the same kind of cells as the original cancer. A lot of cancers grow into solid tumours. However, other types of cancer, such as leukemia, start in blood cells or other cells in the body. On the other hand, benign tumors, those that do not contain cancer cells, do not invade adjacent tissues, nor metastasize to distant organs, and once removed, do not grow back. However, in the brain area it can be life-threatening (Bakshi, et al 2024).

**Cancer Treatment**

Radiotherapy and surgery are the most effective treatments against local cancer, but they are insufficient when it spreads throughout the body. For the treatment of metastatic cancers, chemotherapy, hormonal and biological therapies are more effective; they have the ability to reach most of the body's tissues through the circulatory system. Side effects vary depending on the chemotherapy agent and the most common are: nausea and vomiting, alopecia, anemia, immunosuppression, diarrhea or constipation (Debela et al., 2021). The search for more effective and targeted cancer treatments was made necessary by the toxicity of chemotherapy drugs and the nonspecific destruction of cells; gene therapy emerged as one of the most promising avenues. The vast majority (76.1%) of gene therapy clinical studies conducted to date have focused on hereditary monogenic illnesses (11.1%) and cancer (65.0%), with the latter exhibiting the highest level of success in gene therapy to date (Li et al 2021).

Broadly speaking, gene therapy uses the transfer of a nucleic acid sequence into a patient's cells. Transfer can be carried out by methods involving a viral or non-viral vector and the genetic material can be a gene, typically in the form of cDNA that encodes a functional protein capable of altering a cellular process. Gene therapy is a promising and innovative technique, since genes act as therapeutic agents. It is defined as the transfer of therapeutic genes into an individual's cells to achieve a beneficial effect for patients. Gene therapy was first used as therapeutic intent in 1990 for the treatment of severe combined immunodeficiency ADA-SCID and, later, for severe combined immunodeficiency SCID-X1. Although the results were not very encouraging at the time, it has been demonstrated that human gene therapy is viable and can be useful for the treatment of genetic diseases, for which strategies have been developed for the treatment of complex diseases such as cancer (Belete et al, 2021). The protocol developed was relatively successful, with no side effects and one of the children was practically cured of the abnormality, and since then, experience with gene therapy has grown considerably.

The purpose of gene therapy is to replace a mutated or non-functional gene (causing the disease) in a cell, with the aim of restoring its function (Lejman, et al 2021). Thus, the therapy consists of 3 elements: the genetic material to be transferred, the transfer method or vector and the type of cell where it will be incorporated. TNAs are delivered safely and effectively into the target cell, allowing cancer treatment without harm to healthy cells.

In its beginnings it focused on the treatment of hereditary diseases, and currently its clinical use is limited or under study for various pathologies, with the majority of clinical trials directed at the treatment of cancer, some of which will be discussed in more depth later.

Gene therapy involves the alteration of genetic material through the use of viral vectors (such as adenovirus, retrovirus, lentivirus, and adeno-associated virus) and non-viral vectors (such as lysosomes and naked DNA). The vector is the means that will allow the genetic material to be introduced into the patient.

Technologies and studies have made gene therapy possible both in vivo (the vector is injected into the patient directly, and the cells are modified to obtain the therapeutic effect) and ex vivo (transduction of the cells outside, then introduced into the patient, therefore, is not administered directly) having the transgene not integrated or integrated into the genome. Figure 1 shows the necessary steps that a vector must

The key step in gene therapy is based on a safe, effective and controllable vector. Consequently, viral vectors have demonstrated greater efficiency, but have a disadvantage, immunogenicity. In this sense, non-viral vectors are safer when we treat with in vivo therapy, despite their lower efficiency. Adeno-associated viruses (AAV) have the ability to infect all types of cells, and keep the transferred gene stable. The use of AAV has advantages over other types of viruses. For example, they do not cause disease in humans, which gives them a better safety profile. Furthermore, they have the capacity to infect a wide variety of organs, therefore, they can be adapted to a wide variety of applications in gene therapy. Retroviral and lentiviral vectors have the same function; however, they have drawbacks. Retroviral vectors cause genotoxicity, while lentivirals are less likely to cause mutagenic effects (Naso et al., 2021).

Germ cell gene therapy, on the other hand, is based on genetically modifying germ cells, therefore causing its therapeutic effects to be maintained in the offspring. Currently, it is not allowed due to ethical and legal problems associated with its use in germ lines (Rubeis et al., 2018).

Depending on the application mode, Gene transfer into tumor cells can be performed in vivo or ex vivo. Ex vivo involves harvesting tumor cells from the host, introduction of the therapeutic gene into the cell culture, genetic modification, and reintroduction of the transfected cells into the original tissue.

The in vivo approach involves the transfer of therapeutic genes in situ into tumor cells, systemically or pre-systemically, depending on the location and progression of the tumor (Rosenblum et al., 2018). In vivo therapy is more suitable for cancer treatments than ex vivo.
therapy, since the latter is less invasive and does not require cell proliferation. In the case of administration of naked genes via systemic or presystolic routes, it may be prevented by biological barriers, phagocyte recruitment, renal clearance and stimulation of the immune response. Therefore, the use of stable vectors bypasses the immune system and facilitates targeting of tumor cells. \textit{Ex vivo} therapy requires the proliferation of transfected cells, unlike gene therapy, which aims to inhibit tumor progression by inhibiting cell division. However, \textit{ex vivo} approaches are important in indirect therapies based on immune genes. An advantage of this therapy is the possibility of using non-viral vectors (Bulcha, et al., 2021).

After a search in PubMed, using the words “gene therapy, cancer”, you can see how the number of publications has been increasing in the last 5 years. The 21st century has revolutionized medicine and with it the improvement of techniques such as gene therapy in oncological therapeutic profiles.

Gene therapy is a very promising technology, it represents a revolution in the way we approach the fight against cancer. Their real hope is to cure the cause of the disease at the genomic level. Despite traditional treatments, patients' prognosis has not improved significantly. This is how a new branch of medicine emerges, gene therapy, which promises great achievements in the future. For all this, the importance of carrying out this bibliographic review is justified, with the objective of knowing the different strategies, new research, preclinical and clinical trials along with the results that are being obtained, the limitations that may arise and the advances that are being made are being carried out in the difference countries (Belete et al, 2021). Within this therapy, some strategies have shown very promising results in recent years, such as CAR-T cell therapy in acute lymphoblastic leukemia, oncolytic virotherapy in melanoma or the CRISPR/Cas9 system in solid tumors.

Various forms of cancer have been treated using gene therapy, but further research is necessary to determine the most effective methods for implementing cancer-targeted gene therapy. The objective of this study is to analyse existing literature to determine the primary benefits and potential obstacles associated with the application of gene therapy in cancer treatment.

\textbf{Methodology}

This is a qualitative narrative literature review. The chosen articles were analysed by the author using a critical and subjective approach, employing broad inquiries regarding the topic. A narrative review does not necessitate systematic protocols and explicit criteria for the search and analysis of data, as its purpose is to provide a broad overview of knowledge through the author's subjective intervention in the literature (Paré, 2017). The research was carried out by collecting data on the topic in articles published and accessed in the different databases including Google Scholar, Scopus, PubMed, ResearchGate etc. Contextualization will be used as the inclusion criterion to decide which articles will be referenced and used to explain the subject. Articles that discuss different strategies for using gene therapy to treat cancer will be specifically included. Articles that do not align with the theme or depart from the period under analysis will be returned without consideration. Books, theses, and dissertations will not be included in our analysis, which will only look at indexed scientific articles. A reverse search was performed by looking through the bibliographies of other articles, and databases like PubMed and Google Scholar were used to conduct the bibliographic review up until January 2024. Some types of gene therapy, such as CAR-T therapy, have currently achieved great advances. In this research, the analysis and interpretation of the results were carried out based on the selected articles, which addressed the given topic, carrying out discussions and observations on the use of gene therapy in the treatment of cancer. Tables will be created for analysis and interpretation, which make it possible to monitor and organize the study objectives.
Results and Discussion

Gene therapy represents a viable therapeutic option to conventional medications for the management of genetic illnesses related to metabolism, acquisition, infection, or heredity. Depending on the cells that are targeted by each sort of gene therapy, we can distinguish between them (Chandler, 2016). There have been 24 approved gene therapies worldwide up until 2023, including two for cancer (Daniel Chancellor, 2023). Somatic therapy has been the clinical protocol used in gene therapy procedures; germline therapy is not permitted in any nation since it would result in children who would benefit the rest of the population, who would not benefit from the therapy.

Current Molecular Strategies in Cancer Gene Therapy

Currently, various clinical trials are being developed for different types of cancer: ovarian, breast, lung, prostate, renal cells, melanoma, myeloid leukemia, lymphomas and neuroblastoma. Gene therapy strategies are based on the design of nucleic acids, whether DNA, RNA or RNA interference, which correct molecular alterations that cause cancer.

Gene therapy allows a variety of possibilities to complement conventional treatments and provide new treatment strategies. There are strategies involved in the elimination of tumor cells (cancer suppressor gene therapy), gene silencing (RNA interference as the most used technique), suicidal gene therapy and oncolytic therapy, inhibition of proliferation (antitumor angiogenesis), increase in activity antineoplastic cells of the immune system (immunization gene therapy) and gene editing technique (CRISPR/Cas).

Cancer suppressive therapy: Cancer suppressive therapy involves a strategy to induce the expression of a tumor suppressor gene, achieving apoptosis and death of tumor cells that have that mutated gene. For example, replacement therapy for p53, a tumor suppressor gene (Xia et al 2020).

Oncolytic therapy: Oncolytic therapy has as its function viral replication, oncolysis, and release of virions to neighbouring tumor cells, maintaining said cycle as long as tumor cells that resist infection are still present (Montaño-Samaniego et al., 2020).

Suicidal gene therapy: Suicidal gene therapy consists of the introduction of suicidal genes that encode proteins capable of triggering the death of cancer cells. It can be direct, that is, a gene that encodes a cytotoxic protein that induces cell death when expressed within it, or indirect, where the sensitivity of the tumor to chemotherapy is increased by introducing a gene that expresses an enzyme responsible for catalyse the transformation of a prodrug into a toxic substance, with the capacity to diffuse and eliminate tumor cells around it, without entering systemic circulation or generating side effects.

Presently, the gene therapy methods employed encompass cytokine gene therapy, tumour vaccine therapy, and chimeric antigen receptor T cell (CAR-T) immunotherapeutic therapy.

- **Cytokine gene therapy:** Clinical trials target cytokine genes as activators of the immune response. Cytokines, such as IL-2, IL-12 or interferons, among others, can promote tumor regression by stimulating the immune system. For example, IL-2 gene therapy has been shown to be safe for the treatment of canine mammary cell tumors (Watanabe 1996).

- **Tumor vaccine therapy:** Once the patient's tumor and dendritic cells (DC) are isolated, the vaccine is prepared by transferring genes encoded by tumor antigens into the dendritic cells, and these are reinjected into the patient, activating T cells and inducing specific antitumor immunity. According to research, in prostate cancer, PSA adeno-associated viruses are used to transfer the encoded genes and activate the immune system (Fan, 2023).

- **Chimeric antigen receptor (CAR) T cell immunotherapy:** The main focus of this review study is on looking at Chimeric Antigen Receptor (CAR) T cells, which have a lot of potential for improving treatment methods that use genetically modified T cells. In addition, we look at how these cells are currently used in medicine. CAR-T cell therapies have been in the
works for more than twenty years. Genetically modified T cells from the patient's own body or from a donor are used in this treatment method to fight cancer. CARs are genetically modified receptors that are meant to direct lymphocytes, especially T cells, to find and kill cells that show a certain target antigen. The chimeric antigen receptor (CAR) does its job by attaching to certain antigens on the outside of cells. The Human Leukocyte Antigen (HLA) receptor does not change this activity, which is very important. As a result, it strongly activates T cells and makes the immune system fight the tumour very well (Fischer, 2021). It was proven to work by the US Food and Drug Administration's approval of anti-CD19 CAR-T cell therapy in 2017. This treatment is specifically designed to target B cell malignancies in blood cancers. Using this information, the goal of this review study was to explain how cell-mediated therapies and CAR-T cell therapy have changed over time (Sterner, 2021).

CRISPR/Cas9 technology

Genome editing is making changes or arrangements in specified areas on the DNA. Genome editing, designed to make targeted changes in specialized DNA sections in the genomes of cells, is a technique used to change the DNA within the cell efficiently and error-free. In studies on the genome editing system, ZFN and TALEN systems were the most well-known and used methods. However, in 2013, many studies were conducted on the bacterial adaptive immune system, defined as the CRISPR/Cas system, and the systematic structure that contains small guide RNA instead of DNA binding protein (Cho et al., 2014).

Genome editing tools used before CRISPR were difficult to use, time-consuming and costly to implement. Repairing DNA double strand breaks, gene deletions, gene insertions, point mutations and rearrangement of chromosomes are common features of programmable nuclease such as ZFN, TALEN and CRISPR (Mali et al., 2013). In addition to being used for gene regulation in cultured mammalian cells, ZFN and TALEN are also used to modify the endogenous gene system in organisms such as viruses, bacteria, nematodes (Gaj et al., 2013). TALENs are similar to ZFN in structure, but the ability to design TALENs according to any DNA sequence is seen as an advantage compared to other nucleases. The only difficulty in designing TALENs is that they require thymine nucleotide, which is recognized by folds containing two amino-terminal cryptic repeats at the 5’ end of the target sequence (Ozyigit et al., 2021).

Trials in Gene Therapy

To date, more than 2,600 gene therapy clinical trials have been carried out in the world (Belete et al 2021), the vast majority are focused on cancer treatment, with 67.4%, and in second place are monogenic diseases, with 11.6%.

Currently Most Used Therapies

CAR T cell therapy: Acute lymphoblastic leukaemia (ALL)

Acute lymphoblastic leukaemia (ALL) is a form of leukaemia characterised by an excessive amount of lymphocytes in both the peripheral blood and bone marrow (as shown in figure 2). The potential for metastasis exists to the lymph nodes, liver, spleen, central nervous system, and other organs. (Altmann, 2005).

Figure 2. Acute Lymphoblastic Leukaemia Tumor Development

Incidence, morbidity and conventional therapy

ALL can affect both children and adults, and is the most diagnosed cancer in children, with a
better prognosis than the adult population. Prior to the development of CAR T-cell treatment, the 5-year survival rate for adults and children with R/R ALL was 10% and 21%, respectively. Nonetheless, the treatment has produced noteworthy outcomes, with a high percentage of 57 to 93% full remission (Marzal-Alfaro et al., 2021).

Chemotherapy treatment is administered orally, intravenously and intracathecally and includes high-dose corticosteroid therapy, combined with chemotherapy, depending on the type of treatment. The main substances used in chemotherapy are vincristine, anthracyclines, asparaginase, aracitin, methotrexate, cyclophosphamide and 6-mercaptopurine.

Allogeneic hematopoietic stem cell transplantation is specifically indicated for cases of hypodiploid or resistant forms of acute lymphoblastic leukaemia (ALL). Approximately 10-15% of individuals experience relapses, at which point they undergo allogeneic bone marrow transplants. Patients who are younger than one year old and young adults between the ages of 15 and 25 experience negative outcomes when undergoing intense chemotherapy due to its toxic nature. Immunotherapy has created targeted therapies, such as blinatumomab, which is a bispecific monoclonal antibody. This antibody uses an anti-CD19 antibody to recognise leukemic B cells and a T cell to specifically attack and eliminate tumour cells (Rouger-Gaudichon et al., 2021).

According to Agarwal et al. (2019), cancer immunotherapy research focuses primarily on chimeric antigen receptor (CAR) T cell therapy. Gene therapy is also extremely popular. CAR-T cell therapy works by altering the genes of a patient's T cells, allowing them to fight cancer. As part of this immunotherapy, T cells are genetically modified outside of living things to produce chimeric antigen receptors (CARs) that only recognise surface antigens on tumour cells. After that, these CAR-T cells are expanded, selected based on their needs, and programmed to produce the medicine that will be administered to the patient. Patients are first assessed by the oncology team to ascertain whether the therapy is appropriate and safe. Among other requirements, patients who are eligible for the therapy must have CAR target-positive malignancies (such as CD19), a sufficient quantity of T cells for harvesting, and no active, uncontrollable infection.

The T lymphocytes from the patient are then extracted via leukapheresis. It is common practice to alter the patient's course of treatment in order to obtain more T cells. For example, delivering rescue chemotherapy or temporarily discontinuing corticosteroids prior to leukapheresis. The acquired cells are frozen and sent to a facility for further processing, depending on the type of clinical experiment done. The T cell activation process is the third phase. At this point, the segregated T cells are cultured and activated in vitro to promote CAR production (Dai et al 2016).

The fourth step in the genetic alteration method involves inserting a CAR-expressing gene that targets a specific tumour antigen into T cells. According to Agarwal et al. (2019), lentivirals are the most common and efficient form of viral vector used for gene transfer, permanently modifying the T cell genome and generating human CD19-CAR T cells that target CD8+ cells \textit{in vivo}. These vectors have limited immunogenicity and low oncogenic potential. Other expression systems are currently being analysed.

In the fifth step, different types of culture media are used to grow the cells in a lab dish so that there are enough CAR-T cells. The samples are taken out so that quality control tests can be done on the whole product. Finally, the CAR-T cells are cleaned, gathered, and frozen so they can be sent to the infusion site and given to the patient.

In the days before the CAR T cell infusion, the patient undergoes lymphodepletion chemotherapy to reduce the endogenous lymphocytes or immunosuppressive cells that may threaten, allowing for better extension of CAR T cells, as well as decreasing the tumor burden, thus achieving greater efficacy and less toxicity (Brundo, 2018).
One of the negative consequences is cytokine release syndrome. According to Frey (2017), CRS is a systemic inflammatory response that starts with flu-like symptoms and fever and can lead to potentially fatal hypotension and heart failure. It is associated with the activation and growth of anti-CD19 CAR T cells. Low oxygen intake the toxicity of CRS is also attributed to the immunological response that underpins its efficacy.

CRS prevention can be more effectively controlled with a fractional dosing schedule of CAR T cells, as seen in the trial by Frey et al. (2020).

Neurotoxicity. Neurological problems occur during the first weeks of treatment and include encephalopathies, deficits, cerebral edema and seizures. Risk factors are unclear, but disease burden and severe CRS may increase CNS toxicity. According to studies, CAR T cells rapidly cross the blood-brain barrier and are detected in the cerebrospinal fluid (Xu et al., 2021). The pathogenesis of neurotoxicity may be multifactorial, and is under active investigation (Frey, 2017). Figure 3 depicts conventional CAR T cell administration, which includes patient screening, enrollment, and apheresis for T cell collection, CAR T cell production, pretreatment, and infusion.

Solid tumors
Chimeric antigen receptor T (CAR-T) cell immunotherapies have facilitated the advancement of gene therapies for both haematological and solid cancers. The CAR molecule specifically binds to surface antigens expressed on cancer cells. It specifically interacts with proteins, carbohydrates, and glycolipids. Hence, the choice of the antigen plays a crucial role in determining its potential. The fundamental factors in screening for CAR-T treatment include coverage, specificity, and stability. A high level of antigen coverage suggests that the antigen is present in most of the tumour cells that need to be eliminated. Consequently, this would result in a high level of effectiveness in clearing the tumour. Specificity is assessed by evaluating cytotoxicity beyond the tumour, while stability is determined by measuring the duration of response and the likelihood of recurrence. To enhance coverage and specificity when a perfect target is not available, researchers have developed various strategies, with the most widely used being the combination of targets (Wei et al., 2019). The primary barriers to effective therapy are the dearth of appropriate targets and the milieu that shields solid tumors from the immune system (Titov et al., 2020).

Oncolytic therapy: Melanoma

Incidence, morbidity and conventional therapy
The prevalence of melanoma in Europe is less than 10-25 cases per 100,000 individuals annually, while in Australia it ranges from 50 to
60 cases per 100,000 individuals, and in the United States it ranges from 20 to 30 cases per 100,000 individuals. Anticipate a rise in instances in the forthcoming years, thus it is imperative to enhance early detection and preventative measures for this form of cancer. Ultraviolet radiation, particularly during early childhood, is the primary risk factor of utmost significance (Majem et al., 2021). Prophylaxis is crucial, and consistent application of sunscreen decreases the occurrence of cutaneous melanoma. Regarding local tumours, excisional biopsy is recommended for any suspicious lesion or wound. Following the pathological diagnosis, a surgical procedure is carried out with extensive margins. In cases of advanced melanoma where surgical or local radiotherapy treatments are ineffective, chemotherapy drugs or immunotherapy are employed as alternative options.

Treatment of advanced melanoma consists of immunotherapy or targeted therapy. (Majem et al., 2021). Alternatively, there is a form of gene therapy called Talimogene Laherparepvec, which is an intraluminal virotherapy. This treatment has shown a significant and long-lasting positive response rate, as well as improved overall survival and control in patients with unresectable stage melanoma. T-VEC is a virus established from herpes simplex type 1 and its role is being studied in the treatment of this disease. It is the first intraluminal oncolytic therapy approved in Europe, Australia and the US to treat unresectable stage IIIC, IIIB, or IVM1a melanoma in Europe. It is an ideal treatment option due to its low toxicity profile, especially for elderly patients or patients with multiple comorbidities (Majem et al., 2021).

Anticancer therapies with viruses can be vaccines or oncolytic virotherapies. Vaccines encode and express specific and exogenous tumor antigens, while virotherapies lyse tumor cells to release endogenous antigens. Oncolytic viruses induce both antitumor and antiviral immunity, due to their mechanism of action. There are many clinical trials with oncolytic viruses. An advantage of oncolytic recombinant viruses is that they have the capacity to cause tumor cytoreduction and adaptive immune responses against antigens (Forbes et al., 2018).

**Immunomodulators expressed by oncolytic viruses**

OVs are also vectors of genes that encode immunomodulators whose purpose is to improve the antitumor immune response. The expression of cytokines, such as GM-CSF, IL-2, IL-15, IL-12, INF-a, INF-y or INF-b shows antitumor properties in studies. In the case of melanoma, genes encoding GM-CSF have been used to be administered through an OV, HSV-1 (T-VEC therapy) (Forbes et al., 2018).

**CRISPR technique**

**CRIPS, a novel finding**

Cas9, a revolutionary endonuclease-based genome editing tool with RNA domains, has been created for cancer treatment in recent years. which, due to its ease of use and shorter targeting periods, has dramatically enhanced gene expression and therapy. It boasts a high level of efficiency and precision. Traditional DNA domain binding approaches have inspired the design of new cancer models and therapeutic trials, however due to Because of its high time consumption and intricacy, its application has been severely limited. CRISPR/Cas9 is part of the third generation of gene editing technologies. Since its discovery, numerous researchers have expressed an interest in it. This technology has grown rapidly in recent years and is now widely used in a variety of industries, most notably healthcare. The breakthrough of CRISPR/Cas-9 technology opens up new opportunities for human gene editing. Its rapid progress in recent years has resulted in extensive application in a range of fields, particularly those concerned with genetic illnesses, finding and preventing hazardous germs, treating tumours, and altering plants and animals (Minjiang Chen et al., 2019).

**CRISPR as gene therapy**

The CRISPR/Cas9 system consists of a Cas9 DNA endonuclease and a single guide RNA (sgRNA) that monitors the activity of the RNA in prokaryotes. Just like in bacteria, the Cas protein needs to be capable of recognising the PAM sequence. Figure 4 illustrates how the
guide RNA directs the endonuclease to the specific site where the double-stranded DNA sequence has to be cleaved.

After Cas9 latches onto and chops the responsible DNA sequence, a healthy copy will be inserted to replace the aberrant one. Despite being more error-prone than other types of recombination, NHEJ (non-homologous end joining) is the most commonly used method for gene therapy, despite the fact that HDR (homology-directed repair) is the most efficient approach.

Potential for application against cancer:

The CRISPR/Cas9 technique may be a novel and promising strategy to correct oncogenic aberrations, given that cancer is a genetic disease derived from these cumulative aberrations.

- **Epi genome editing of tumor cells.** The elimination of genes involved in the growth and survival of tumor cells promotes apoptosis, inhibiting tumor growth.
- **Fight against infection by carcinogenic viruses.** Viral oncogenes can be directly removed by a specific Cas9-sgRNA from their genome, including genes for virus replication. All of this contributes to suppression of expression and induction of cancer cell death.
- **Therapy directed at the stroma.** It is used to reprogram the tumor stroma and achieve carcinogenic effects.
- **Development of anti-cancer drugs.** CRISPR technology makes it possible to validate and identify drug targets and resistance genes, and to find new drug targets thanks to its high efficiency. This facilitates the development of anti-cancer drugs.
- **Immunotherapy against cancer:** CRISPR technology improves the effectiveness of T cell therapy.
- **Oncolytic virotherapy:** Certain viruses can be genetically altered using gene editing techniques to enhance their ability to reproduce within the host, thereby triggering immune responses that combat cancer.

**Most treated types of cancer**

Compared with non-solid tumors such as leukemia, solid tumors such as breast, liver, lung, prostate, and colorectal cancer showed worse progress in gene therapy-based treatments (Abu Hazafa et al., 2020). Therefore, the development of CRISPR/Cas9 improved the situation. Tests carried out demonstrate that CRISPR/Cas9 can inhibit tumor growth by inhibiting proliferation and metastasis and inducing apoptosis.

On the other hand, the efficacy of CAR-T cell therapy against solid tumors remains limited. Therefore, the researchers developed another potential approach, CRISPR/Cas9-engineered CAR-T cells ex vivo, which holds great promise for cancer immunotherapy.

For example, the PD-1 receptor regulates T cell activity. Systemic inhibition of the PD-1 gene via electroporation of the CRISPR/Cas plasmid into T cells can enhance cytotoxicity against cancer cells. As a result, genetic knockout of PDCD1 can increase the activity of human T cells (Jennifer R. Hamilton 2020). Clinical trials have looked into genome editing using CRISPR/Cas9 systems to improve the longevity of T cell activity. It is now difficult to achieve high ex vivo gene editing efficiency through viral vectors or electroporation. The most common method is electroporation, which is highly effective at transferring CRISPR systems to T cells, B cells, and NK cells.

In recent years, a first phase I trial of edited CRISPR/Cas9 PD-1 T cells have been carried out in patients with advanced non-small cell lung cancer (NCT02793856) (Hazafa et al., 2020).
Breast cancer is one of the main causes of death in women, and accounts for 30% of diagnoses worldwide. There are several subtypes of cancer associated with the estrogen receptor (ER), which are luminal A, luminal B, triple negative and Her2-enriched breast cancer. Luminal subtypes are characterized by being the most lethal, and TNBC by high metastasis, where the chemokine CXCL12 and the CXR4 and CXR7 receptors play an important role. The incidence of TNBC is much lower, around 15%, and the only therapeutic option is chemotherapy, where the cell lines are more sensitive specifically to three drugs, docetaxel, gemcitabine and doxorubicin.

Recently, the CRISPR/Cas9 technique has proven to be an effective therapeutic target in breast cancer. A study was carried out where the CRISPR/Cas9 technique was used for the joint and independent elimination of the CXR4 and CXR7 genes in the TNBC cell line MDAMB - 231. According to the results, co-deletion of these genes was shown to inhibit the proliferation, migration and invasion of TNBC cells much more effectively.

Conventional treatments for Colon cancer (Montaño-Samaniego et al., 2020) are anti-EGFR and anti-VEGF antitumor agents, but they are limited to a metastatic setting and have narrow efficacy. In recent years, Li et al. performed an in vitro assay, demonstrating that the introduction of a Cas9/sgRNA system in the CaCO2 cell line inactivated the function of Par3L in CRC cells. Par3L inhibits the Lkb1/AMPK pathway, allowing the survival of CRC cells, since Lkb1 is a tumor suppressor protein and AMPK regulates cell metabolism and apoptosis. Thus, the elimination of Par3L inhibited proliferation and promoted apoptosis in CRC cells.

Furthermore, Par3L knockout cells are more sensitive to antitumor therapies, considering them a good target for CRISPR/Cas9 technology (Shojaei Baghini et al., 2022).

A large percentage of the mortality associated with cancer is due to its ability to spread and produce metastasis. Identifying the genes associated with metastasis could be very useful for researchers, since by focusing anticancer therapies in these centers, metastasis could be slowed down and even prevented.

### Other types of cancer and therapies used

In recent years, gene transfer technologies have progressed considerably (Table 1). This wide variety of molecular strategies, together with delivery vectors, are being used to establish new treatments and complement conventional therapies, significantly improving their therapeutic effect.

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Source: Montaño-Samaniego et al 2020

To treat patients with prostate cancer, Freytag et al. (2007) looked into and documented the effectiveness of adenovirus-mediated gene therapy in conjunction with intensity-modulated radiation therapy. Prostate biopsies performed after treatment showed that only 22% of the patients assessed tested positive for adenocarcinoma, which is less than the predicted >40%. The group of patients who got the intervention showed improvement when the prognosis was assessed. The trial's technique
allowed participants with prostate cancer to achieve superior radiation results. NIU et al. (2016) investigated the therapeutic resistance of triple-negative breast cancer (TNBC) to doxorubicin when anti-miRNA-181 gene therapy was used. The study found that genotoxic therapies increased the expression of miRNA-181 in TNBC cells. Basuony et al (2020) worked to find a new gene therapy for oral squamous cell cancer by stopping the growth of cancer-causing miRNA-221 with an inhibitor. Three methods were used in the study: MTT test, flow cytometry, and cell death. It was found that anti-miRNA-221 plays a major role in cell death and suppression. Using the RT-PCR method, it was seen that miRNA-221 was significantly decreased, along with DKNIB/p27 being increased and EGFR being decreased, but this difference was not statistically significant when compared to the negative control.

**Conclusion**

Gene therapy is aimed at reinforcing and promoting neoplastic self-destruction through different pathways. Within it, CAR-T cell therapies have already achieved excellent results in blood-related cancers, such as lymphoma and leukemia, achieving long-lasting remission.

On the other hand, oncolytic therapy has shown good results, as has targeted gene inactivation (knockout) using CRISPR-Cas9, which has improved the action of T cells, enhancing the application of the therapy to more types of cancer. The discovery of CRISPR proteins has largely contributed to the great leap forward in gene therapy.

Regarding r/r ALL, toxicity and efficacy must be optimized; for this, fractionated dosing using CTL019 is the most promising technique, optimizing safety and preserving efficacy. Despite their immunogenicity, viral vectors are used more than non-viral vectors, especially in in vivo therapy, such as CAR-T cell therapy, because they facilitate reaching the site of action. On the other hand, the cost of the therapy means that its use is limited to those cases where its application is only feasible. For this reason, I would consider a restructuring of the production of vital importance, especially of therapies with CAR-T cells and CRISPR-Cas9 delivery systems, which manage to reduce costs and be able to modulate the dose based on the characteristics of the individual, and thus be able to increase the accessibility of these treatments to the majority of patients and institutions.

It is evident that advances in recent years have improved the survival of patients who would otherwise die very early. However, much remains to be investigated to offer treatment schemes that not only improve survival, but also the quality of life of these patients.

Although there is not a large volume of clinical studies that ensure the use of gene therapy, the studies already carried out demonstrate its potential to revolutionize conventional cancer treatments, especially when pharmacological or surgical interventions cause discomfort and often do not produce good results. It is expected that over the next few decades they will be learned, regulated and improved, so that gene therapies bring great hope in the prognosis and quality of life of cancer patients.

**References**


Fretyag, S. O., Movsas, B., Aref, I., Stricker, H., Peabody, J., Pegg, J., Zhang, Y., Barton, K. N.,


