Bölüm 11

Recombinant DNA Technology in Medical Perspective-Genetic Engineering Applications

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Abstract

Recombinant DNA technology encompasses the precise regulation of target gene expression for therapeutic purposes. This technology employs a strategic approach involving gene products that hold promise for the treatment and prevention of various diseases. The utilization of target proteins enables the safe, cost-effective, and scalable production of therapeutics. The interdisciplinary collaboration among fields such as genetic engineering, gene therapy, and biotechnology further enriches this technology. Overcoming initial reservations, recombinant drugs have now gained widespread acceptance and rapid commercial approval. The extensive applications of recombinant DNA technology extend to bioremediation as well as the treatment of severe diseases through gene therapy and genetic modification techniques. This book chapter aims to provide an in-depth exploration of the diverse range of applications and rapidly evolving techniques within the field of recombinant DNA technology, with a specific focus on medical applications categorized by product types and targeted diseases.

Recombinant DNA Technology Basic Principles

Recombinant DNA technology encompasses the transfer of a specific DNA fragment, containing the target gene region, from one organism to another via a vector. Consequently, the recipient organism, altered through molecular cloning, is regarded as genetically modified. Transgenic host organisms receive foreign DNA originating from a distinct species. The manipulation of genes, including the introduction of mutations or the suppression of gene expression, using recombinant DNA vectors is referred to as gene targeting.

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Collectively, these principles fall within the domain of Genetic Engineering. Genetic engineering entails the modification of an organism's genotype by employing recombinant DNA technology to achieve desired genetic traits (Khan S, 2016).

Recombinant DNA technology involves the extraction of the desired DNA fragment from the host organism's cell and subsequent insertion into a vector, such as a phage or plasmid. These vectors facilitate the expression of the pertinent gene product within the recipient cell. The organism that receives the transfer of recombinant DNA is characterized as a genetically modified organism (GMO) (Fig 1).

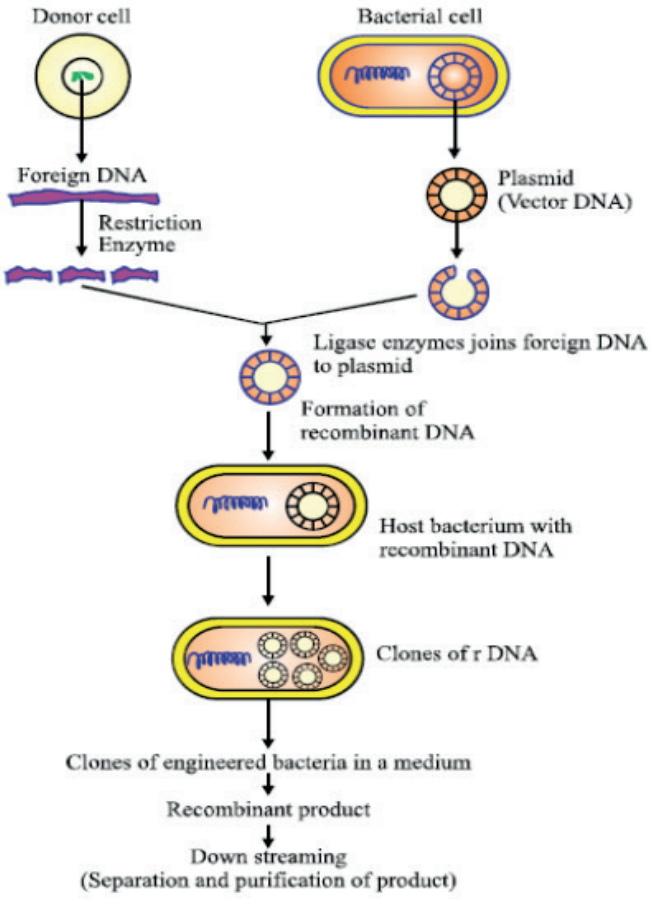

Figure 1. Recombinant DNA technology general process (Vedantu, 2023).

In the past century, the concept of controlling the expression of target genes through recombinant DNA technology seemed like a distant dream for improving disease traits. However, recent advancements in this field have yielded remarkable effects in advancing human life. This technology now enables the safe, cost-effective, and efficient production of essential proteins for health and nutrition purposes. It has the potential to address crucial aspects of human existence, including enhancing health, improving food resources, and bolstering resilience against adverse environmental influences (Lomedico PT, 1982). Global health concerns have become a daily focus due to multiple factors such as shifting Earth conditions, population growth, climate change, transportation density, the rapid spread of infectious diseases, the proliferation of toxic compounds, and their lasting impacts on human health. Recombinant DNA technology plays a pivotal role in improving health conditions through the development of novel vaccines, drugs, diagnostic kits, monitoring devices, and therapeutic approaches. Notable examples of genetic engineering in the health domain include the synthesis of synthetic human insulin and erythropoietin using genetically modified bacteria, as well as the creation of experimental mutant mice for research purposes (Galambos L, 1998). Recombinant DNA technology has opened up new avenues for innovation by genetically modifying microorganisms, animals, and plants to obtain medicinal substances. This approach has led to the production of a wide range of therapeutic products with immediate impact in medical genetics and biomedicine (Galambos L, 1998). Biotechnological drugs, which are predominantly recombinant in nature, target various diseases such as Kaposi's sarcoma, leukemia, colorectal cancer, kidney cancer, ovarian cancer, hereditary disorders (cystic fibrosis, familial hypercholesterolemia, Gaucher disease, hemophilia A, severe combined immunodeficiency disease, and Turner syndrome), as well as conditions like diabetic foot ulcers, diphtheria, genital warts, hepatitis B, hepatitis C, human growth hormone deficiency, and multiple sclerosis (Liu W, 2013). Our lives and health face significant risks due to water and food scarcity, general hygiene issues, uncontrolled microbial flora, emerging viral threats, and the proliferation of new, potentially deadly diseases. In this era where traditional health strategies fall short, the products and strategies derived from the fields of recombinant DNA technology, genetic engineering, and biotechnology offer new opportunities to safeguard general health (Liu W, 2013).

Gene therapy is a process wherein genetic material is transferred to a specific target cell with the aim of achieving a clinical benefit (Stribley JM, 2002). Through gene transfer techniques, introduced DNA sequences can

intervene in gene function, restore lost function, or initiate novel functions. Previously, gene therapy was primarily associated with the treatment of single-gene inherited disorders. However, it is now recognized that approximately 3,000 medical disorders are caused by alterations in a single gene (Sachs BP, 1993). The continuously expanding literature in the field of gene therapy, coupled with advancements in scientific understanding, holds promise for the treatment of numerous hereditary and chronic diseases. In contemporary times, as our knowledge deepens and technology progresses, there is growing recognition that the treatment of many of these conditions may indeed be feasible. This is particularly true in situations where suitable genes and target tissues can be readily identified, and an appropriate method for gene transfer is employed. These developments inspire optimism regarding the potential for effective interventions in the management and treatment of various hereditary and chronic diseases.

Gene therapy delivery comprises three distinct steps: administration, delivery, and expression (Sachs BP, 1993). Administration pertains to the introduction of DNA into the body, while delivery involves the translocation of genetic material from the site of application to the nucleus of the target cell. The bioavailability of the gene to the target cell, gene uptake, and intracellular trafficking influence the success of gene therapy delivery. Finally, expression entails the production of the therapeutic gene product within the cell (Ledley FD, 1999; Leiden JM. 1996). Effective gene therapy necessitates the combination of a suitable disease target with an appropriate gene delivery system, resulting in long-term therapeutic outcomes with minimal or no toxicity (Yaron Y,1997).

Recombinant DNA Technology and Gene Therapy

Recombinant DNA technology is a scientific discipline encompassing the process of introducing genes from one species into the DNA of a distinct organism (Fig 2). A significant breakthrough in the field of gene therapy was achieved through the Human Genome Project. Initiated by the National Human Genome Research Institute, which was established by the American National Institutes of Health (NIH) on October 1, 1989, the project aimed to comprehensively identify the approximately 30,000 genes encoded within the 46 human chromosomes (Stribley JM, 2002).

The continuous advancements in molecular biology technology, combined with an enhancedunderstanding of molecular intricacies, have revealed the substantial potential of gene therapy. Furthermore, the recognition that more than 40,000 known human diseases possess a genetic component amplifies

the significant role gene therapy can play in addressing complex medical conditions (Vile R, 1994).

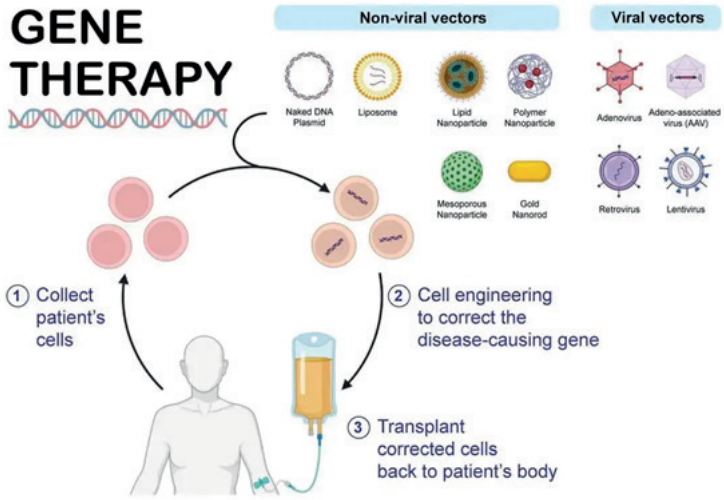

Figure 2. The concept of ex-vivo gene therapy (RSscience, 2023).

Current strategies for gene therapy

Given the vast array of human genetic diseases, a diverse range of gene therapy strategies is required, as a one-size-fits-all approach is not viable. Gene therapy holds potential for treating genetic disorders such as inborn errors of metabolism, cancer, and viral infections where a genetic cause has been identified. Consequently, prior to establishing a protocol, a thorough investigation into the biology of the target disease and the target cell population is essential (Lindsten JE, 1991).

The intended outcome or benefit of gene therapy protocols may vary, and can be categorized as either corrective or cytotoxic. Corrective gene therapy primarily focuses on inherited disorders wherein the genetic defect manifests in a specific cell type, aiming to transfer the necessary genes to rectify the genetic abnormality. On the other hand, cytotoxic gene therapy seeks to directly eliminate abnormal cells by producing toxic proteins. While originally conceived as a strategy to combat malignant tumors, cytotoxic gene therapy also applies to benign tumors. The therapeutic gene may encode a product that converts a non-toxic prodrug into a toxic chemical within the transfected cells, leading to the death of the targeted cell and surrounding bystander cells. Only cells that have received the gene are able to metabolize the drug, resulting in cell lysis (Morgan RA, 2006).

Promising genes for gene therapy often encode enzymes that act upon a prodrug, converting it into a toxic metabolite. These genes are frequently employed to induce cell death specifically in tumor cells, and thus, this approach is referred to as "suicide" gene therapy.In addition to the classification of gene therapy as corrective or cytotoxic, approaches can also be defined based on the desired duration of therapy. While gene therapy often appears to bring about a permanent alteration requiring stable function of both the cell and the transfected gene, transient gene expression is often sufficient. Examples of situations where transient gene expression is suitable include recombinant DNA vaccines or tumor immunotherapy, where long-term correction is not necessary (Patyar S, 2010). Recombinant DNA technology exhibits a wide range of applications in the treatment of diseases and the improvement of health conditions, encompassing various therapeutic approaches (Lam P, 2013).

Gene therapy

Gene therapy has emerged as an advanced and promising technique for the treatment and prevention of various diseases. Notably, it has made significant strides in the field of immunotherapy, with metastatic melanoma being recognized as the first successful study in this area. This milestone has played a pivotal role in establishing the effectiveness of gene therapy in this particular field. In the treatment of genetic diseases, gene therapy has been employed to increase the expression of specific proteins and combined with immunotherapy for therapeutic purposes (Restifo NP, 2012).

Cancer, a leading cause of mortality worldwide, is characterized by the uncontrolled and invasive growth of cells that can potentially spread to other parts of the body through the bloodstream and lymphatic system, known as metastasis. Primary treatment modalities for cancer include surgery, conventional chemotherapy, and radiotherapy. However, despite the high efficacy of these treatment approaches, they do not yield significant improvements in nearly half of cancer patients, highlighting the necessity for novel strategies in cancer treatment (Kershaw MH, 2013).

Significant advancements have been made in the field of gene therapy for hereditary diseases (Fig 3), with promising studies focusing on the treatment of hemophilia A (HA). HA is a bleeding disorder caused by mutations in the F8 gene, which encodes clotting factor VIII (FVIII). Current treatment options typically involve regular infusions of FVIII concentrates throughout a patient's lifetime. However, alternative approaches utilizing viral gene

therapies that directly deliver F8 in vivo have shown early success, offering hope for improved treatment outcomes (Ginn SL, 2012).

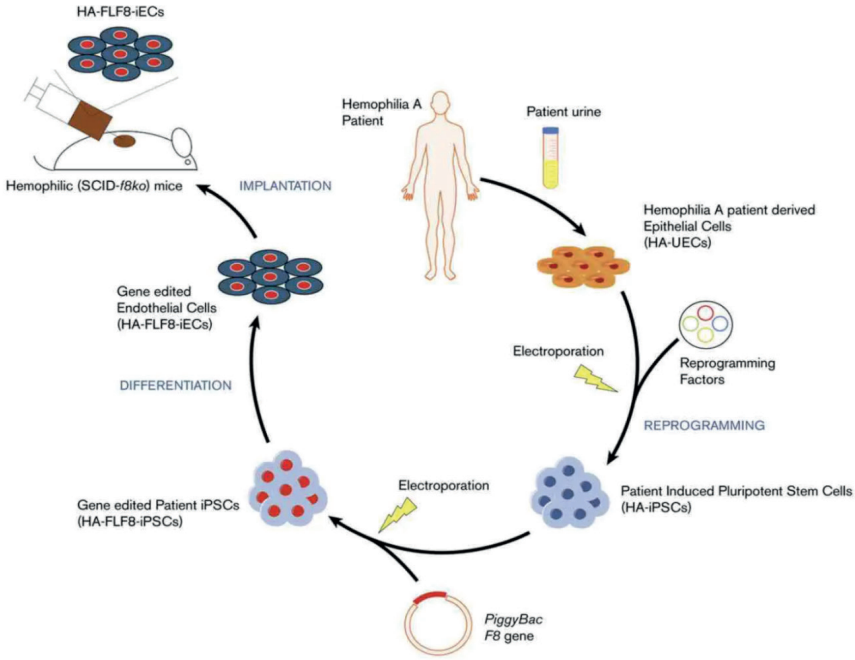

Figure 3. A research combining iPSC and ex vivo gene therapy to treat hemophilia (Ginn SL, 2012).

The importance of developing strategies in gene therapy and recombinant DNA studies cannot be overstated. However, it is worth acknowledging that these technologies are not easily accessible to everyone, primarily due to their high cost. Therefore, pioneering and advanced literature studies play a crucial role in advancing our understanding and application of these techniques. In the subsequent sections, innovative approaches in the field of health within the realm of gene therapy and recombinant DNA technology will be discussed, providing a broad overview without delving into intricate study details.

Immunotherapy-Recombinant Vaccines

Immunotherapy, specifically using recombinant vaccines, has emerged as an effective alternative approach in cancer treatment. This therapy involves the development of personalized recombinant cells or products designed to stimulate the immune system and overcome the tolerance induced by cancer

cells. The primary objective is to enhance antigen presentation, facilitate the migration of immune cells to lymphoid and tumor tissues, and impact effector cells. Examples of such studies include cancer vaccines, specifically engineered cytotoxic T cells, and immunocytokines (Mellman I, 2011).

The growing interest in gene therapy and the establishment of a solid literature in this field have enabled a more active role in cancer treatment (Moschella F, 2010). Successful identification of tumor-expressed antigens and associated vasculature, as well as guiding T cells to the appropriate targets prior to their transfer to patients, are key operative points in gene engineering (Fig 4). Consequently, uniquely designed gene-engineered T cells can be utilized for the immunotherapy of metastatic cancer (Amerikan Cancer Society, 2012).

Cancer cells often evade immune system detection, suppressing the survival and infiltration of T cells. However, such challenges can be addressed through recombinant DNA studies (22).

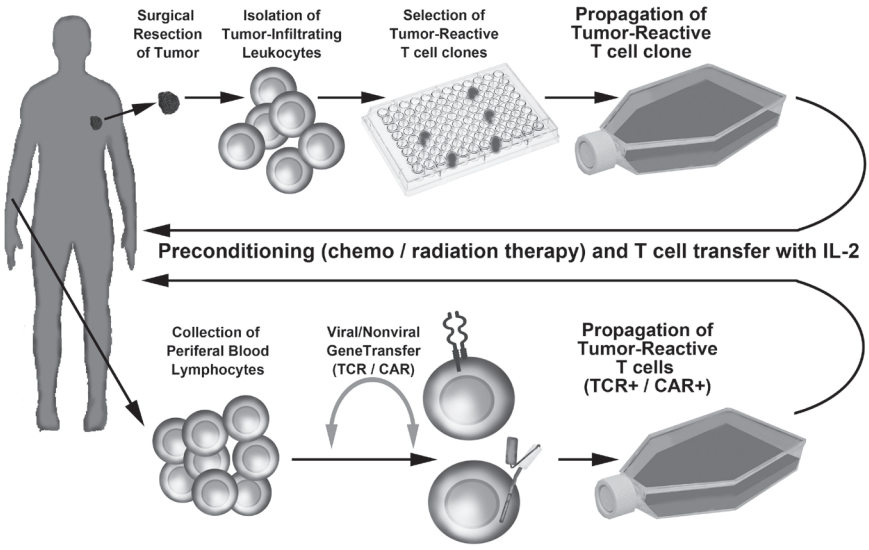

Figure 4. Clinical application of the T cell-mediated tumor immunotherapy. Diagram on the top depicts application of the Tumor-Infiltrating Lymphocytes (TILs). Diagram on the bottom illustrates application of the genetically engineered (TCR and CAR-modified) T cells (Huynh G, 2013).

Genetic engineering processes hold promise for treating various types of cancer, including gynecological, skin, neurological, hematological malignancies, cardiovascular diseases, and even pediatric tumors. Approaches such as the addition of tumor suppressor genes to immunotherapy, oncolytic virotherapy, and gene-directed enzyme prodrug therapy have been explored. Notably, the tumor suppressor gene p53 has played a significant role in cancer treatment efforts. Combining p53 gene transfer with chemotherapy or radiotherapy has been shown to be effective (Coventry BJ, 2012).

The first recombinant drugs produced in this field were interferon-α2a (IFN-α2a) and interferon-α2b (IFN-α2b). These drugs were approved by the FDA in 1986 and are indicated for conditions such as hairy cell leukemia, Kaposi's sarcoma, chronic myeloid leukemia, malignant melanoma, and follicular lymphoma (the last two cancers are treated with IFN-α2b only). They are produced from *Escherichia coli* strains. Although these drugs exhibit low response rates and can be toxic at high doses, they have shown favorable survival outcomes in a select group of patients with a predisposition to autoimmunity (Coventry BJ, 2012). Another recombinant drug approved by the FDA is interleukin-2, which is derived from *Escherichia coli* metabolism (Eriksson F, 2008). Interleukin-2 stimulates the production and expansion of T cells, and it is particularly effective in treating metastatic renal cell carcinoma and metastatic melanoma. It serves as an active immunotherapy agent when combined with cancer vaccines, as most vaccines aim to elicit or enhance an immune response against tumor-specific antigens through cytotoxic T lymphocytes (CTLs), which directly target and eliminate malignant cells (Eriksson F, 2008).

Recombinant Vaccines

The ideal target for cancer vaccines are Tumor-specific antigens (TSAs), which are essential for tumor formation and cancer progression. Tumor-associated antigens (TAAs) are not the right target because they can be found in tumors of the same histology as well as in tumors of different origin and even some normal cells and are not as effective as TSAs, triggering a weak immunological response (Eriksson F, 2008; Dillman RO, 2011).

Recombinant vaccines are produced in genetically engineered systems and are therapeutic agents with advantages such as being more specific, safer, more biocompatible, and anti-allergic than conventional products (Bele T, 2012). These vaccines include recombinant live (viral and/or bacterial) vector vaccines, nucleic acid (DNA and/or RNA replicon) vaccines, protein and peptide vaccines, viral-like particle (VLP) vaccines, whole-cell vaccines

(DC- or tumor cell-based)), edible vaccines, and combined approaches (eg, prime-boost vaccination) (Vergati M, 2010).

Cancer vaccines may also be based on single proteins or protein combinations, including heat shock proteins, peptides, anti-idiotype antibodies, and fusion proteins. The faster production, storage and transportation of these vaccines, whose costs vary with the target and technological infrastructure, are important advantages (Mäkelä PH, 2000).

Vector-based vaccines rely primarily on viruses, bacteria, or yeasts to introduce recombinant genes, such as genes expressing TAAs, cytokines, or costimulatory molecules, into APCs (Bolhassani A, 2009). This method stimulates APCs to produce an immune response against the tumor. There are advantages and disadvantages for each type of vector, but in general these vectors allow for the inclusion of all tumor antigen gene/fragments and infecting APCs (Fig 5). Commonly used major vectors include *Saccharomycescerevisiae*, *Salmonella*, vacciniavirus, adenovirus. Some vectors may cause an immunological reaction against them, which can be eliminated by a detailed preliminary study (Palena C, 2006).

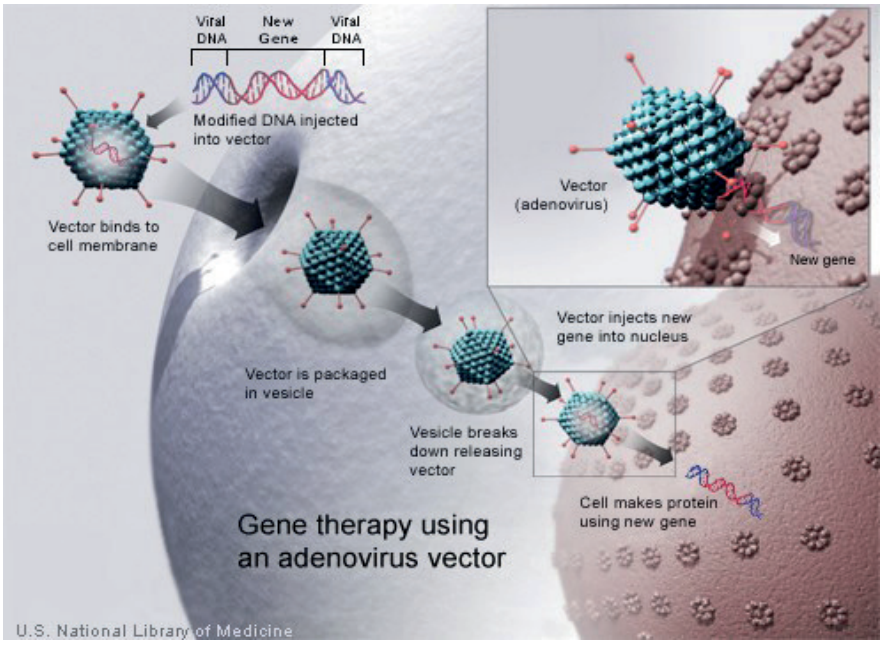

Figure 5. Gene therapy using an adenovirus vector (National Library of Medicine, 2023).

DNA vaccines function by introducing DNA encoding protein antigens into immune cells using vectors. They have the disadvantage of low immunogenicity. However, they have important advantages, such as long-lasting transgene expression without the need for booster vaccinations, the activation of both cell-mediated and humoral immune responses, and lower production costs compared to other vaccine types (Gulley JL, 2010; Aldrich JF, 2010; Schlom J, 2012).

Cancer vaccines can be categorized as prophylactic vaccines, which prevent the transmission of cancer-causing viruses and the development of cancer in high-risk individuals, and therapeutic vaccines, which target existing cancer. An example of a successful prophylactic vaccine is the human papillomavirus (HPV) vaccine. The identification of etiological agents, such as the hepatitis B virus causing hepatocellular carcinoma, is a major focus in the development of these vaccines (Bharadwaj M, 2009; Bolhassani A, 2011).

Therapeutic vaccines aim to stimulate antigen-specific T cells and reprogram memory T cells. However, engineering therapeutic vaccines can encounter challenges, including incomplete knowledge of tumor pathophysiology and variations in immune responses to antigens (Palucka K, 2011; Mellman I, 2011).

Antibodies

Antibodies have emerged as powerful therapeutic agents in the treatment of cancer, inflammation, and infectious diseases, representing the next generation of therapeutic tools. While some antibodies enhance the immune system, others exert their effects independently of the immune response. The primary function of antibodies is to target specific components of cancer cells, halting their growth and inducing cell death (Aldrich JF, 2010).

Monoclonal antibodies (mAbs), a product of recombinant DNA technology, are the most well-known class of antibodies. Their successful utilization in cancer therapy exemplifies the potential of this approach in treating various types of cancer, even when employed in isolation from other immune system components (Dillman RO, 2003; Hansel TT, 2010).

Next-generation recombinant antibodies function similarly to naturally occurring immunoglobulins and serve as effective agents in treating cancer patients by mimicking the immune response mediated by antibodies (Bono JS, 2002). These antibodies target a range of proteins, including unique tumor neoantigens, epidermal growth factor receptor (EGFR), vascular

endothelial growth factor (VEGF), human epidermal growth factor receptor 2 (HER2/neu), and specific antigens such as cytotoxic T-lymphocyte antigen (Reiter Y, 2001; Bono JS, 2002; Forero A, 2003). Notable clinical successes of recombinant humanized therapeutic antibodies have been observed in the improvement of overall survival and time to disease progression in the treatment of various human malignancies, including breast, colon, and hematological cancers (Zeng Y, 2018; Korpela H, 2021).

Recombinant immunotoxins

The combination of knowledge regarding the expression of antigens on cancer cells and the remarkable advancements in recombinant DNA technology and antibody engineering has led to the development of highly effective therapeutic agents known as recombinant immunotoxins. These immunotoxins consist of a potent protein toxin fused to a targeting fragment, such as a recombinant antibody fragment or growth factor. By binding to specific surface antigens present on cancer cells, these molecules induce cell death through the catalytic inhibition of protein synthesis.

Recombinant immunotoxins have been extensively investigated for their therapeutic potential against solid tumors and hematological malignancies. They have undergone rigorous characterization for their biological activity in vitro on tumor cell lines and in vivo in animal models of human tumor xenografts. Notably, recombinant immunotoxins have demonstrated remarkable efficacy both in laboratory settings and in animal models, particularly against malignant cancers that are resistant to conventional treatment modalities such as surgery, radiation, and chemotherapy (Bafati C, 2020).

Gene therapy-Genetic engineering Applications in Cardiovascular Diseases

Cardiovascular diseases, as the second leading cause of mortality after cancer, have become a priority area for genetic engineering applications. Among the thousands of inherited diseases identified, over 100 monogenic hereditary cardiovascular diseases have been identified to date (Ylä-Herttuala S, 2017). These cardiovascular diseases, such as Marfan syndrome and familial pulmonary hypertension, follow Mendelian genetic laws and are caused by mutations in single genes. Clinical trials and animal experiments have demonstrated the potential of gene editing technology in treating single-gene diseases. Applying gene editing technology to prevent and treat cardiovascular diseases, particularly congenital arterial diseases, has

become a focal point of current cardiovascular research and will shape future therapeutic approaches.

Heart failure, a group of diseases affecting 64.3 million individuals worldwide, exhibits increased incidence and prevalence with advancing age (Karakikes I, 2012). Preclinical studies have shown that cardiac gene therapy can be an effective treatment strategy (Ishikawa Y, 1994). The goal of gene therapy is to achieve adequate expression of the transferred therapeutic gene in the targeted organ. Additionally, current therapeutic agents have been utilized in heart failure treatment, targeting abnormalities in calcium processing, beta-adrenergic signaling, or promoting blood vessel growth through therapeutic angiogenesis. Another approach explored in gene therapy studies for coronary artery disease (CAD) is enhancing perfusion in hypoxic regions of the heart (Wirth T, 2013).

Common vectors employed in recombinant DNA technology include adenoviruses (which exhibit short gene expression times), adeno-associated viruses (AAV), lentiviruses, retroviruses, and plasmids (which allow for long gene expression times). Factors such as production conditions, safety, biocompatibility, non-toxicity, non-allergenicity, and ecological compatibility are crucial considerations when selecting a vector (Hedman M, 2009). In cardiac gene therapy, delivery can be achieved through intramyocardial injections or intravascular infusions. Intramyocardial injections can be performed via a transthoracic approach or an endovascular approach using a catheter. Intravascular infusions can be conducted antegradely via the coronary arteries or retrogradely via the coronary vessels. These newly developed methods are currently being applied in experimental animal models in preclinical studies.

Therapeutic angiogenesis

The human heart relies on terminal arteries for its blood supply. However, in chronic ischemic conditions, collateral arteries can develop to bypass occluded branches and provide blood flow to ischemic regions (Fig 6). Therapeutic angiogenesis aims to mimic and enhance this natural process of collateral formation, thereby improving blood supply to hibernating myocardium. Gene therapy offers advantages over direct protein-mediated approaches by providing local, long-lasting, and high-levelexpressionoftherapeutic agents

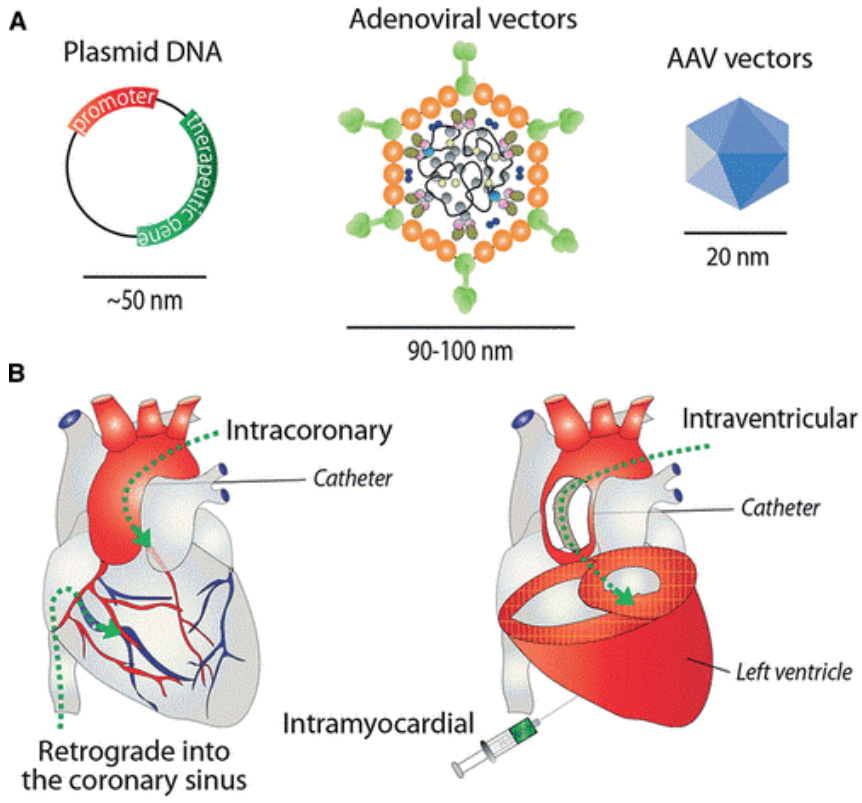

Figure 6. Delivery vehicles and routes for cardiac gene therapy (Cannatà A, 2020).

Initially, plasmids were the vectors of choice in gene therapy due to their low immunogenicity and reliable production process. However, their transfection efficiency in the heart is limited (Müller OJ, 2007; Rincon MY, 2015).

Adenoviruses and adeno-associated viruses (AAVs) have been the primary viral vectors used in cardiac clinical trials. Adenoviruses can efficiently transduce cardiomyocytes and have relatively short gene expression times, making them suitable for diseases that would benefit from the short-term effects of angiogenesis in clinical practice (Stolberg SG, 1999). However, the high rate of adenoviral gene transfer can elicit immune responses that may be harmful to the host (Rosengart TK, 2013). Various studies have reported the safety of using low doses of adenoviruses (Hedman M, 2009; Hartikainen J, 2017).

Heart failure etiology is not fully understood and poses challenges for pharmacological approaches. Therefore, gene therapy holds significant therapeutic potential in this field.

Potential uses of gene therapy in obstetrics

Gene therapy in obstetrics presents unique advantages compared to other fields. The opportunity for intrauterine treatment of fetal pathology, which encompasses congenital malformations and genetic diseases, is considered a remarkable advancement. Corrective genetic interventions during the antepartum period offer significant theoretical benefits over postpartum treatment of congenital diseases. Antepartum treatment has demonstrated high efficacy in severe conditions like Lesch-Nyhan syndrome and lipid storage diseases, whereas postpartum treatment typically provides only palliative care (Holzinger A, 1995).

Fetal gene therapy holds great promise in realizing the goal of a healthy generation. Advancements in technologies related to in vitro fertilization (IVF) and embryo transfer enable pre-implantation diagnosis of certain hereditary disorders. A comprehensive understanding of disease etiology allows for the identification of specific mutations that may impact the fetus and the extent to which they do so (Alexander H, 1988).

Fetal gene therapy not only stands out as the most intriguing approach within the field of gene therapy in healthcare, but also poses significant ethical considerations. The primary objective should be to conduct studies that adhere to ethical and moral values, with a focus on treating congenital malformations and genetic diseases rather than pursuing traits for so-called "designer babies." In the context of genetic diseases, this therapy may involve the incorporation of hereditary artificial chromosomes through micromanipulation of the preimplantation embryo. Naturally, such approaches will bring about substantial changes in the concepts of the genome and fertility treatment. Germ-line gene therapy, which involves modifying the genome, can have individual, familial, and societal implications. Approaches lacking good intentions raise significant ethical concerns.

Mesenchymal stem cells (MSCs) have garnered considerable attention in the field of regenerative medicine and immunotherapy due to their unique properties. Among MSCs, amniotic fluid stem cells (AFS) have emerged as a particularly promising substrate for pioneering tissue engineering and cell replacement strategies in perinatal medicine. This is especially relevant in diseases such as birth asphyxia, preterm birth, and congenital abnormalities, where interventions can be performed during pregnancy, allowing for the

collection of amniotic fluid cell samples and subsequent research. Cultivating AFS in vitro during pregnancy enables their potential use in postnatal applications.

Hypoxic ischemic encephalopathy (HIE) and myelomeningocele (MMC) are debilitating conditions that lead to permanent neurological disabilities, and current treatment options are severely limited. However, experiments conducted on animal models of HIE and MMC, as well as human clinical trials, have demonstrated the beneficial effects of MSCs, including AFS, on the central nervous system through paracrine mechanisms. These findings suggest that autologous AFS therapy may hold promise as a viable treatment option for intractable neurologic diseases in the perinatal period, including HIE and MMC (Daigo O, 2018).

The unique properties of AFS, combined with the ability to access and culture these cells during pregnancy, offer a valuable opportunity to explore innovative therapeutic approaches. By harnessing the regenerative potential of AFS and their paracrine effects on the central nervous system, novel interventions can be developed to address the unmet medical needs of infants affected by HIE and MMC. Continued research and clinical investigations are crucial to further unravel the therapeutic potential of AFS in perinatal medicine and advance the field of regenerative medicine towards more effective and personalized treatments for neurologic conditions (Fig 7).

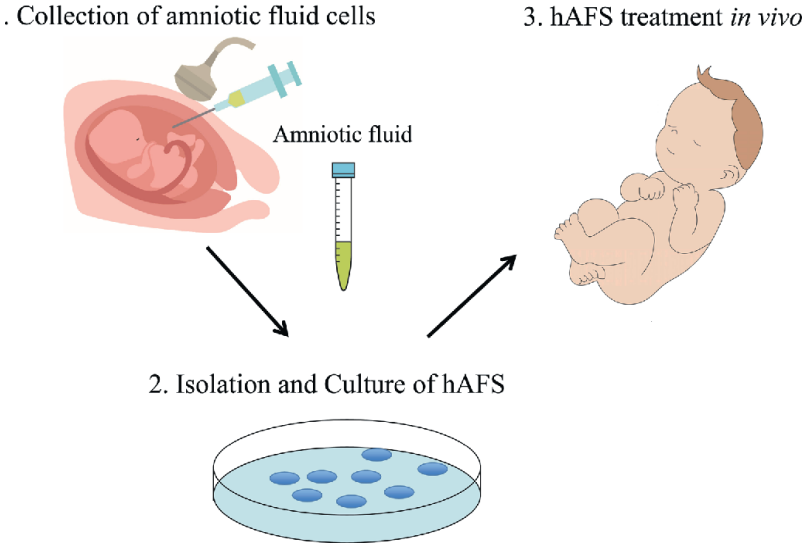

Figure 7. Autologous AFS therapy for perinatal disease (Daigo O, 2018).

Gynecological oncology and potential uses of gene therapy in gynecology

The fields of gynecology and gynecological oncology, which were traditionally focused on surgical interventions, have now emerged as pioneering areas in gene therapy research. Gynecological oncology, in particular, has shown great potential for gene therapy applications (Wivel NA, 1998). Various possibilities for gene therapy are being explored in this field, including immunotherapy, gene transfer for drug resistance, transfer of tumor suppressor genes to cancer cells, and cytotoxic gene therapy. Immunotherapy, exemplified by tumor "vaccines," has been successfully applied in gynecological oncology, especially in the context of human papillomavirus (HPV)-associated cervical carcinomas (He Y, 1997).

The majority of gene therapy studies in gynecological oncology have focused on breast cancer and ovarian carcinoma. Breast cancer, the most common malignancy in women, affects a significant number of women in the USA and is responsible for 20% of female cancer-related deaths each year. Genetic studies have revealed the presence of specific oncogenes and loss of tumor suppressor genes, which contribute to reduced survival rates. These findings serve as the foundation for current gene therapy strategies in breast cancer (Giannios J, 1997).

Ovarian cancer, the leading cause of death among gynecological malignancies, is often diagnosed at an advanced stage with poor long-term survival rates. Gene therapy has shown promising results in the treatment of ovarian cancer (Fig 8). Animal studies employing cytotoxic gene therapy have provided important survival data, particularly in addressing chemotherapy resistance, a significant challenge in ovarian carcinoma treatment (Barnes MN, 1997). Consequently, ovarian carcinoma studies are among the most advanced in terms of human clinical trials in gene therapy.

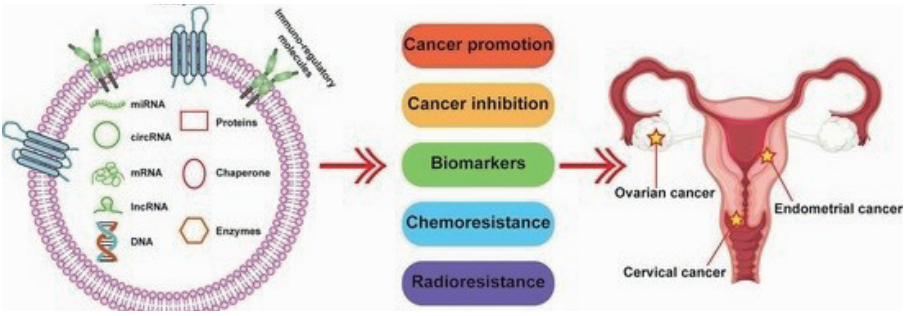

Figure 8. Application of extracellular vesicles in gynecologic cancer treatment (Renwen Z, 2022).

Similar approaches are being explored in the earlier stages of research for endometrial carcinoma. In vitro studies using human endometrial carcinoma cell lines have demonstrated successful application of the hsv-tk/ganciclovir protocol with the p53 adenoviral vector (Rosenfeld ME, 1996; Al-Hendy A, 2000), as well as positive results in an in vivo animal model (Vandier D, 2000). This approach involves the use of ganciclovir (GCV) in combination with the "cell suicide gene" HSV1-tk. Cells transfected with HSV1-tk undergo apoptosis upon exposure to GCV, leading to selective elimination of tumor cells (Kunishige I, 1999; Ramondetta L, 2000). GCV has already been approved by the FDA for antiviral therapy against cytomegalovirus infections.

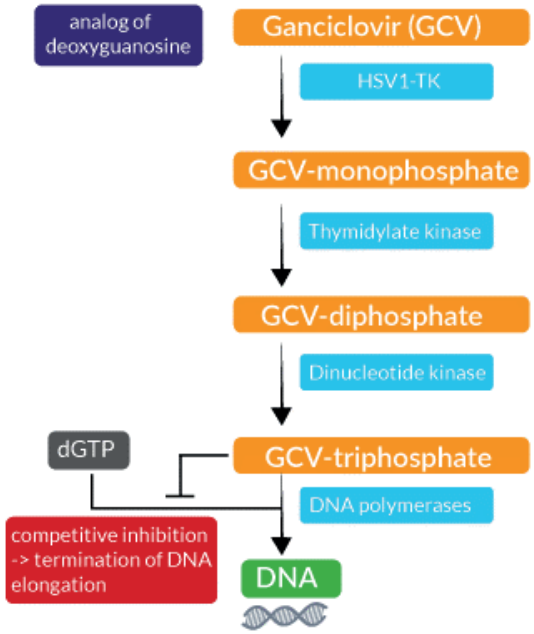

Figure 9. Mechanism of action of ganciclovir (Chang CM, 2013).

Gene therapy also holds potential for treating benign gynecological conditions. Uterine leiomyomas, benign tumors originating from uterine smooth muscle, affect more than 30% of women of reproductive age and often lead to menorrhagia and pelvic pressure symptoms (Fig 9). Gene therapy offers a non-surgical treatment approach for these tumors. Successful application of the hsv-tk/ganciclovir protocol has been reported in cytotoxic

gene therapy strategies aimed at shrinking uterine leiomyomas in vivo (Niu H, 1998).

Overall, the potential applications of gene therapy in gynecology, gynecological oncology, and obstetrics offer a range of strategies not only for malignant tumors but also for benign diseases. With advancements in gene therapy agents and strategies, these fields have the potential to revolutionize clinical practice and improve patient outcomes.

Conclusion

The disciplines of Biotechnology, Recombinant DNA technology, and Genetic Engineering hold immense therapeutic potential in the field of healthcare. However, it is essential to acknowledge the critical intersection of science and ethics in these areas. Technological advancements are influenced by an ethical framework that is shaped by the prevailing state of technology. While these technologies were initially developed with the primary goal of promoting general well-being in healthcare, they also carry the potential for unintended consequences that can adversely affect individuals and society. Therefore, it is imperative to thoroughly examine the ethical implications of proposed developments.

Technologies that prioritize the enhancement of general well-being in health deserve ethical recognition. However, it is important to be mindful of practices that may be driven solely by the desire to achieve treatment at any cost. For instance, the biotechnological treatment of infertility is a particularly contentious area that gives rise to numerous ethical dilemmas. Even in less controversial scenarios such as heart disease or cancer, advancements have created heightened expectations regarding the availability of treatments. Nonetheless, technology, in general, is built upon a strong ethical foundation.

In the face of doubts and concerns, it is not prudent to outright reject technology. Doing so would imply a level of contentment with the existing levels of hunger, disease, and deprivation. Instead, biotechnology and genetic engineering should strive to benefit human lives. Before addressing technical concerns, it is essential to answer the fundamental question of "Why should it be done?" (O'Mathúna DP, 2007). Many concerns surrounding technology are centered on technical and practical aspects. Therefore, it is crucial to differentiate between technological authority and technological necessity as the guiding principles in approaching these issues. Failure to do so may result in the unchecked pursuit of youthfulness, longevity, and perfection at the expense of public health, ultimately leading to genome

changes and unpredictable evolutionary shifts that may do more harm than good to humanity.

In conclusion, the disciplines of Biotechnology, Recombinant DNA technology, and Genetic Engineering possess significant therapeutic potential in healthcare. However, it is paramount to navigate the ethical landscape associated with these fields. Ethical considerations should guide the development and application of these technologies, ensuring that they serve the greater good and contribute to the well-being of individuals and society as a whole.

Referances

Aldrich JF, Lowe DB, Shearer MH, Winn RE, Jumper CA, Kennedy RC. Vaccines and immunotherapeutics for the treatment of malignant disease. Clin Dev Immunol. 2010;2010:697158.

Aldrich JF, Lowe DB, Shearer MH, Winn RE, Jumper CA, Kennedy RC. Vaccines and immunotherapeutics for the treatment of malignant disease. Clin Dev Immunol. 2010;2010:697158.

Alexander H, Weber W. Micromanipulation of gametes and pre-implantation mammalian embryos—a review. Zentralbl Gynakol. 1988:110;745-749

Al-Hendy A, Magliocco AM, Al-Tweigeri T, Braileanu G, Crellin N, Li H, *et al***.** Ovarian cancer gene therapy: repeated treatment with thymidine kinase in an adenovirus vector and ganciclovir improves survival in a novel immunocompetent murine model. Am J Obstet gynecol. 2000;182:553-559

Bafati C, Machado DB, Cislaghi B, Salman OM, Karanikolos M, McKee M, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020; 396: 1204– 22.

Barnes MN, Deshane JS, Rosenfeld M, Siegal GP, Curiel DT, Alvarez RD. Gene therapy and ovarian cancer: a review. Obstet Gynecol. 1997;89:145-155.

Bele T. Treating cancer with vaccine. Webmed Central Immunotherapy. 2012;3:1–7.

Bharadwaj M, Hussain S, Nasare V, Das BC. HPV & HPV vaccination: issues in developing countries. Indian J Med Res. 2009;130:327–33.

Bolhassani A, Mohit E, Rafati S. Different spectra of therapeutic vaccine development against HPV infections. Hum Vaccin. 2009;5:671–89.

Bolhassani A, Safaiyan S, Rafati S. Improvement of different vaccine delivery systems for cancer therapy. Mol Cancer. 2011;10.

Bono JS, Rowinsky EK. The ErbB receptor family: a therapeutic target for cancer. Trends Mol Med. 2002;8(Suppl):S19–26.

Cannatà A, Hashim A, Sinagra G, Giacca M. Gene Therapy for the Heart Lessons Learned and Future Perspectives. Circulation Research. 2020;126:1394–14.

Chang CM. *In vitro* treatment with Ganciclovir restores the functionality of exhausted T cells from cancer patients. Int. J. Gerontol. **2013;***7*(3):171-6.

Coventry BJ, Ashdown ML. The 20th anniversary of interleukin-2 therapy: bimodal role explaining longstanding random induction of complete clinical responses. Cancer Manag Res. 2012;4:215–21.

Daigo O, Hirotaka M, Yushi A, Toshimitsu O, Fukutake M, Tadashi M, Kei M , Mamoru T. Human Amniotic Fluid Stem Cells: Therapeutic

Potential for Perinatal Patients with Intractable Neurological Disease. Keiko J. Med. 2018 Dec 26;67(4):57-66.

Dillman RO. Cancer immunotherapy. Cancer Biother Radiopharm. 2011;26:1–64.

Dillman RO. Monoclonal antibody therapy. In: Oldham RK, Dillman RO ed(s). Principles of Cancer Biotherapy. 4th ed. Dordrecht: Kluwer Academic Publishers, 2003: 329-90.

Eriksson F. Tumor immunology. In: Eriksson F, ed. Novel approaches for cancer immunotherapy. Stockholm: Karolinska Instituet, 2008: 12.

Forero A, Lobuglio AF. History of antibody therapy for non-Hodgkin's lymphoma. Semin Oncol. 2003;30.

Galambos L, Sturchio JL. Pharmaceutical firms and the transition to biotechnology: a study in strategic innovation. *Business History Review*. 1998;72(2):250–278.

Giannios J, Ioannidou-Mouzaka L. Molecular aspects of breast and ovarian cancer. Eur J Gynaecol Oncol. 1997;18:387-393.

Ginn SL, Alexander IE, Edelstein ML, Abedi MR, Wixon J. Gene therapy clinical trials worldwide to 2012. *Journal of Gene Medicine*. 2013;15(2):65–77.

Gulley JL, Arlen PM, Hodge JW, Schlom J. Vaccines and Immunostimulants. In: Hong WK et al, eds. Holland Frei Cancer Medicine. 8th ed. Shelton, Connecticut: People's Medical Publishing House, 2010: 725-36.

Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJT. The safety and side effects of monoclonal antibodies. Nat Rev Drug Discov. 2010;9:325–38.

Hartikainen J, Hassinen I, Hedman A, Kivelä A, Saraste A, Knuuti J, *et al*. Adenoviral intramyocardial VEGF-DDNDC gene transfer increases myocardial perfusion reserve in refractory angina patients: A phase I/IIa study with 1-year follow-up. Eur Heart J. 2017; 38: 2547– 2552.

He Y, Huang L. Growth inhibition of human papillomavirus 16 DNA-positive mouse tumor by antisense RNA transcribed from U6 promoter. Cancer Res. 1997;57:3993-3999.

Hedman M, Muona K, Hedman A, Kivelä A, Syvänne M, Eränen J, *et al*. Eight-year safety follow-up of coronary artery disease patients after local intracoronary VEGF gene transfer. Gene Ther. 2009; 16: 629– 34.

Hedman M, Muona K, Hedman A, Kivelä A, Syvänne M, Eränen J, et al. Eight-year safety follow-up of coronary artery disease patients after local intracoronary VEGF gene transfer. Gene Ther. 2009; 16: 629– 34.

Holzinger A, Trapnell BC, Weaver TE, Whitsett JA, Iwamoto HS. Intraamniotic administration of an adenoviral vector for gene transfer to fetal sheep and mouse tissues. Pediatr Res. 1995;38: 844-850.

http://www.cancer.org/acs/groups/cid/documents/webcontent/003013-pdf.pdf.(2012).

Huynh G. TD. Melanoma-From Early Detection to Treatment. 2013. DOI10.5772/50853. ISBN978-953-51-0961-7.

Immunotherapy. American Cancer Society.

Ishikawa Y, Sorota S, Kiuchi K, Shannon RP, Komamura K, Katsushika S, *et al*. Downregulation of adenylyl cyclase types V and VI mRNA levels in pacing- induced heart failure in dogs. J Clin Invest. 1994; 93: 2224– 9.

Karakikes I, Hadri L, Rapti K, Ladage D, Ishikawa K, Tilemann L, *et al*. Concomitant intravenous nitroglycerin with intracoronary delivery of AAV1.SERCA2a enhances gene transfer in porcine hearts. Mol Ther. 2012; 20: 565– 71.

Kershaw MH, Westwood JA, Darcy PK. Gene-engineered T cells for cancer therapy. *Nature Reviews Cancer.* 2013;13(8):525–54

Khan S, Ullah MW, Siddique R, Nabi G, Manan **S**, Muhammad Yousaf M, Hongwei H. , Role of Recombinant DNA Technology to Improve Life. Int J Genomics. 2016; 2016: 2405954.

Korpela H, Järveläinen N, Siimes S, Lampela J, Airaksinen J, Valli K. Gene therapy for ischaemic heart disease and heart failure. Journal of İnternal medicine, 2021;290(3):567-582.

Kunishige I, Samejima Y, Shiki Y, Moriyama A, Meruelo D, Saji F, et al. Suicide gene therapy for human uterine adenocarcinoma cells using herpes simplex virus thymidine kinase. Gynecol Oncol. 1999;72: 16-25.

Lam P, Khan G, Stripecke R, et al. The innovative evolution of cancer gene and cellular therapies. *Cancer Gene Therapy.* 2013;20(3):141–149.

Ledley FD. Nonviral gene therapy: the promise of genes as pharmaceutical products. Hum Gene Ther. 1995;6:1129-1144.

Leiden JM. Gene therapy promise, pitfalls, and prognosis. N Engl J Med.1996; 333: 871-873.

Lindsten JE, Pettersson U. Etiology of human disease at the DNA level, Raven Press, New York. 1991; 143-189.

Liu W, Yuan JS, Stewart CN, Jr. Advanced genetic tools for plant biotechnology. *Nature Reviews Genetics.* 2013;14(11):781–793.

Lomedico PT. Use of recombinant DNA technology to program eukaryotic cells to synthesize rat proinsulin: a rapid expression assay for cloned genes. *Proceedings of the National Academy of Sciences of the United States of America.* 1982;79(19):5798–5802.

Mäkelä PH. Vaccines, coming of age after 200 years. FEMS Microbiol Rev. 2000;24:9–20.

Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480:480–9.

Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480:480–9.

Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science*. 2006;314(5796):126–129.

Moschella F, Proietti E, Capone I, Belardelli F. Combination strategies for enhancing the efficacy of immunotherapy in cancer patients. Ann N Y Acad Sci. 2010;1194:169–78.

Müller OJ, Katus HA, Bekeredjian R. Targeting the heart with gene therapy-optimized gene delivery methods. Cardiovasc Res. 2007; 73: 453– 62.

NIH U.S. National Library of Medicine. 2023.

Niu H, Simari RD, Zimmermann EM, Christman GM. Nonviral vector-mediated thymidine kinase gene transfer and ganciclovir treatment in leiomyoma cells. Obstet Gynecol. 1998;911:735-740.

O Mathúna DP. Bioethics and biotechnology. Cytotechnology. 2007;53(1-3): 113–119.

Palena C, Abrams SI, Schlom J, Hodge JW. Cancer vaccines: preclinical studies and novel strategies. Adv Cancer Res. 2006;95:115–45.

Palucka K, Ueno H, Banchereau J. Recent developments in cancer vaccines. J Immunol. 2011;186:1325–31.

Patyar S, Joshi R, Byrav DS, Prakash A, Medhi B, Das BK. Bacteria in cancer therapy: a novel experimental strategy. J Biomed Sci. 2010;17:21.

Ramondetta L, Mills GB, Burke TW, Wolf JK. Adenovirus-mediated expression of p53 or p21 in a papillary serous endometrial carcinoma cell line (SPEC-2) results in both growth inhibition and apoptotic cell death: potential application of gene therapy to endometrial cancer. Clin Cancer Res. 2000;6: 278-284.

Reiter Y. Recombinant immunotoxins in targeted cancer cell therapy. Adv Cancer Res. 2001;81:93-124.

Renwen Z, Yixing Z.Application of Extracellular Vesicles in Gynecologic Cancer Treatment. *Bioengineering* 2022*;9:*(12) 740.

Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. *Nature Reviews Immunology*. 2012;12(4):269–281.

Rincon MY, VandenDriessche T, Chuah MK. Gene therapy for cardiovascular disease: Advances in vector development, targeting, and delivery for clinical translation. Cardiovasc Res. 2015; 108: 4– 20.

Rosenfeld ME, Wang M, Siegal GP, Alvarez RD, Mikheeva G, Krasnykh V, *et al.* Adenoviral-mediated delivery of herpes simplex virus thymidine kinase results in tumor reduction and prolonged survival in a SCID mouse model of human ovarian carcinoma. J Mol Med. 1996;74: 455-462.

Rosengart TK, Bishawi MM, Halbreiner MS, Fakhoury M, Finnin E, Hollmann C. et al. Long-term follow-up assessment of a phase 1 trial of angiogenic gene therapy using direct intramyocardial administration of an adenoviral vector expressing the VEGF121 cDNA for the treatment of diffuse coronary artery disease. Hum Gene Ther. 2013; 24: 203– 8.

RSscience. Gene Therapy: A Promising Biotechnology for the Treatment of Genetic Diseases and Cancers – Basic Introduction https://rsscience.com/gene-therapy (2023).

Sachs BP, Korf B. The Human Genome Project: implications for the practicing obstetrician. Obstet Gynecol. 1993;81: 458-462.

Schlom J. Therapeutic cancer vaccines: current status and moving forward. J Natl Cancer Inst. 2012;104:599–613.

Stolberg SG. The biotech death of Jesse Gelsinger. NYT M. 1999; 136–140: 149– 50.

Stribley JM, Khurram SR, Hairong N, Christman GM. Gene therapy and reproductive medicine. Fertility and Sterility. 2002 ;2:645-657.

Vandier D, Calvez V, Massade L, Gouyette A, Mickley L, Fojo T, *et al.* Transactivation of the metallothionein promoter in cisplatin-resistant cancer cells: a specific gene therapy strategy. J Natl Cancer Inst. 2000;92: 642-647.

Vedantu Web Site. Mention the role of vectors in recombinant DNA technology. https://www.vedantu.com/question-answer/a-mention-the-role-of-vectors-in-recombinant-dna (2023).

Vergati M, Intrivici C, Huen NY, Schlom J, Tsang KY. Strategies for cancer vaccine development. J Biomed Biotechnol. 2010;2010:1–14. doi: 10.1155/2010/596432.

Vile R, Russell SJ . Gene transfer technologies for the gene therapy of cancer. Gene Ther. 1994:1;88-98.

Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. Gene. 2013; 525: 162– 9.

Wivel NA, Wilson JM. Methods of gene delivery. Hematol Oncol Clin North Am, 12 (1998), pp. 483-501.

Yaron Y, Kramer RL, Johnson MP, Evans MI. Gene therapy. Is the future here yet? Obstet Gynecol Clin North Am. 1997: 24;179-199.

Ylä-Herttuala S, Bridges C, Katz MG, Korpisalo P. Angiogenic gene therapy in cardiovascular diseases: Dream or vision? Eur Heart J. 2017; 38: 1365– 71.

Zeng Y, Li J, Li G, Huang S, Yu W, Zhang Y, et al. Correction of the marfan syndrome pathogenic fbn1 mutation by base editing in human cells and heterozygous embryos. Mol Ther. 2018; 26:2631–7.