https://www.mdpi.com/1999-4923/12/3/233?trk=public_post_main-feed-card-text Novel ways and strategies to combat cancer have been developed in large part thanks to the concept of gene therapy to address its development.Transcription Activator-Like Effector Nucleases (TALENs) Are artificial DNA nucleases formed by fusing a DNA-binding domain with a nonspecific nuclease domain derived from Fok I endonuclease that specifically cut the required DNA sequence.Citation15 TALE effectors DNA-binding domain has a repeating unit of 33-35 conserved amino acids.An ideal vector can administer a gene to a specific tissue, accommodate enough foreign gene size, achieve the level and duration of transgenic expression enough to correct the defect gene, non-immunogenic, and safe.The gRNA unit guides Cas9 to a specific genomic locus via base pairing between the crRNA sequence and the target sequence.Citation22 CRISPR-Cas-mediated gene repair, disruption, insertion, or deletion are thus finding applications in several areas of biomedical research, medicine, agriculture, and biotechnology.Citation22,Citation23 Gene Delivery Technologies Since the emergence of recombinant DNA technology that helps gene-therapy, how to effectively and safely administer gene products is the major challenge.TALEN uses to edit genomes by inducing DSB that cells respond to with repair mechanisms.Citation17,Citation18 CRISPR-Cas CRISPR is a heritable, adaptive immune system of bacteria that provides them with the memory of previous virus infections and defends against re-infection.CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, which are interrupted by "spacer" sequences. These "spacer" sequences are viral sequences integrated during past viral infections when transcribed into short RNA sequences, are capable of guiding the Cas endonuclease to complementary sequences of viral DNA.Viral Vectors Used for Gene Delivery Viruses were the first and the most widely used vectors to administer genes into the target tissue.Once entered, viruses release their genome into the nucleus for viral gene expression.Citation25,Citation26 Herpes simplex virus (HSV), adenovirus (Ad), adeno-associated virus (AAV), and lentivirus (LV) are the most important viral vectors.Citation27,Citation28 Bacterial Mediated Gene Transfer (Bactofection) Some bacteria specifically target tumor cells leading to RNA interference (RNAi) and gene silencing by inhibiting RNA activity, such as protein synthesis.Several in vivo and in vitro studies revealed that intracellular bacteria such as Salmonella spp., Listeria monocytogenes, Shigella flexneri, Bifidobacterium longum, E. coli, and Yersinia enterocolitica use to deliver plasmids pro-drug converting enzymes and cytotoxic agents into the target cell.Citation29 Phase I trial is undergoing by using Listeria, Bifidobacterium, Salmonella, Shigella, and Clostridium gene therapy against cancer.Another clinical trial is ongoing on the effects of Lactococcus synthesizing interleukin 10 against colitis in Phase II.Citation30,Citation31 Chemical-Based Nonviral Vectors Viral-vectors-based gene transfer displays better and long-term gene encoding but has some limitations like immunogenicity, less specific to the target cell, carcinogenicity, high cost and cannot deliver large genome size.Indeed, there are several methods, and most have a similar mode of gene delivery, ie, physically formed transient pores in the cell membrane through which the genetic material enters into the host cell.Citation40,Citation41 Needle and jet injection, hydrodynamic gene transfer, electroporation, sonoporation, magnetofection, and gene gun bombardment are examples of physical DNA delivering methods.Citation42-Citation44Generally, non-viral vectors help to deliver small DNA, large DNA (plasmid DNA), and RNA (Si RNA, m RNA) into the

target tissue.Citation36–Citation38 Physical methods use different mechanical forces to facilitate the administration of gene material into the host tissues.Non-viral methods display better advantages due to relatively safe, can deliver a large genome, and ease for production.Citation32–Citation35 Chemical vectors, also known as non-viral vectors grouped as organic and inorganic vectors.Gene Therapy for Cancer Treatment Cancer occurs due to disrupting the normal cell proliferation and apoptosis process.Advances in cancer therapy need a novel therapeutic agent with novel mode of action, several mechanisms of cell death, and synergy with conventional management.Several gene therapy approaches were developed for the management of cancer, including anti-angiogenic gene therapy, suicide gene therapy, immunotherapy, siRNA therapy, pro-apoptotic gene therapy, oncolytic virotherapy, and gene directed-enzyme prodrug therapy.Citation45 By November 2017, greater than 2597 clinical trials were conducted on gene therapy in the world.Among these trials, greater than 65% are associated with cancer, followed by monogenetic and cardiovascular diseases.Citation8 The use of CAR T cell therapy showed promising results for the management of both myeloid and lymphoid leukemia.Gene therapies possess all these profiles.In order to alter the expression of a gene product or alter the biological characteristics of cells for therapeutic purposes, gene therapy involves introducing foreign genomic material into the host tissue.Reference 1 The drawbacks of using peptides in recombinant medicine, including limited bioavailability, instability, high production costs, clearance rates, and severe toxicity, are addressed via gene therapy.The fundamentals of genome-editing techniques, including meganucleases, zinc finger nucleases, transcription activator-like effector nucleases, and the CRISPR/Cas9 system with its underlying processes, are summed up in this article.Thanks to advancements in gene delivery technology and a better understanding of disease pathophysiology, gene therapy is a successful treatment for a number of disorders.Citation 6, Citation 7: Gene therapy has a wide range of applications, ranging from immunization to gene replacement and knockdown for hereditary disorders such as cancer, hemophilia, hypercholesterolemia, and neurological diseases.Viral (adenoviral, adenoassociation, herpes simplex virus) and nonviral (physical: DNA bombardment, electroporation) and chemical (cationic lipids, cationic polymers) gene transport techniques are also described in this review.Citation9Citation5 Gene therapy involves transferring genetic material (such as DNA or RNA) into the host organ by means of a vector.In vivo gene therapy involves introducing the genetic material into the target organ; ex vivo gene therapy involves altering host cells that are subsequently re-administered.While managing genetic diseases was initially the main goal of gene therapy, it is currently being used to treat a variety of conditions with various patterns of acquired and inherited disorders.A number of molecular approaches have emerged in recent decades that aid in editing DNA codes and modifying mRNA through post-transcriptional alterations.Reference Gene knockdown, deactivating problematic genes, inserting a new gene to treat a condition, and replacing dysfunctional genes with therapeutic genes are some of the ways that gene treatments work.Reference 3 Gene therapy can be applied to germline or somatic cellsGene Editing Tools Conventional gene therapy mostly depends on viral-based delivery of genes that either randomly integrates into the host genome (eg retroviruses) or remains as extrachromosomal DNA copy (eg AAV]) and expresses a protein that is missing or mutated in human disorder.The ZFN-encoding plasmid-based targeted

administration of the required genes decreases the limitations of viral administration.From a clinical viewpoint, HDR is favorable for restoring mutations in genes or for integrating genes for therapeutic purposes.Citation10–Citation13 Currently, there are four different gene-editing nuclease enzymes available based on their structures: meganucleases, zinc-finger nucleases, transcription activator-like effector nucleases, and CRISPR-associated nucleases.ZFN has three zinc fingers that each identifies three base pair DNA sequence to form a three-finger array that attaches to nine base pair target sites and the non-specific cleavage domain.Citation14,Citation15 ZFPs deliver a site-specific DSB to the genome and facilitate local homologous recombination that enhances targeted genome editing.The complexity in re-engineering and low editing efficiency limits the uses of MNs.Citation14 Zinc Finger Nucleases (ZFNs) Artificially produced by fusing site-specific zinc finger protein with the non-specific cleavage domain of the FokI restriction endonuclease.Genome-editing nucleases can be modified to recognize and break the genome at specific DNA sequences, resulting in DSBs, which are efficiently repaired by either NHEJ or HDR.Citation10,Citation11 NHEJ repair damaged DNA without a homologous template.Novel ways and strategies to combat cancer have been developed in large part thanks to the concept of gene therapy to address its development.However, the importance of ex vivo therapy in indirect immunological gene-based therapies (explained in Section 2.7) should not be overlooked.However, persistent proliferative signaling, growth suppressor evasion, resistance to cell death, replicative immortality, deregulation of cellular energetics, promotion of angiogenesis, activation of invasion and metastasis, and avoidance of immune destruction are among the main characteristics shared by tumor cells [3].The interaction between tumor cells and the surrounding environment creates a complex tumor microenvironment (TME) that fosters tumor intra-heterogeneity, with geographically and phenotypically diverse subclones, regardless of the monoclonal origin of the neoplasia [2].These characteristics maintain the basis of a TME that is made up of immune system cells like macrophages, T and B lymphocytes, and natural killer cells, as well as a distinctive extracellular matrix (ECM), cancer-associated fibroblasts (CAFs), mesenchymal stromal cells, endothelial cells, and pericytes (reviewed in [4]).In order to achieve a target gene edition, expression modification of a target gene, mRNA, or synthesis of an exogenous protein, gene therapy involves introducing exogenous nucleic acids, such as genes, gene segments, oligonucleotides, miRNAs, or siRNAs, into cells [8,9,10,11,12,13,14,15,16,17,18,19].Therefore, it is necessary to use stable carriers/vectors that shield the nucleic acid cargo from circulatory nucleases, evade the immune system, and guarantee that the therapeutic vector is efficiently targeted into the tumor cells without dissipating in the body through the lymphatic and blood systems or avoiding non-target cells [21].Since ex vivo approaches require the proliferative advantage of transfected cells, which is antagonistic to the main goals of cancer gene therapeutics, which primarily aim to inhibit the tumor progression by tackling the tumor cell division ability, the in vivo approach is less invasive and more appropriate for treating cancer despite its apparent limitationshttps://www.tandfonline.com/doi/full/10.2147/BTT.S302095#abstract In order to alter the expression of a gene product or alter the biological characteristics of cells for therapeutic purposes, gene therapy involves introducing foreign genomic material into the host tissue.Gene Editing Tools Conventional gene therapy mostly depends on viral-based delivery of genes that either randomly

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processes, are summed up in this article.Finding a method to effectively deliver these effectors to the targeted cell and tissue has proven difficult, despite the abundance of gene modulation techniques, such as gene silencing, antisense treatment, RNA interference, and gene and genome editing.Thanks to advancements in gene delivery technology and a better understanding of disease pathophysiology, gene therapy is a successful treatment for a number of disorders.Citation 6, Citation 7: Gene therapy has a wide range of applications, ranging from immunization to gene replacement and knockdown for hereditary disorders such as cancer, hemophilia, hypercholesterolemia, and neurological diseases.Biological barriers, nuclease susceptibility, phagocyte absorption, renal clearance, and/or immune response stimulation make it difficult to administer bare TNAs in systemic and pre-systemic administrations [23].Viral (adenoviral, adenoassociation, herpes simplex virus) and nonviral (physical: DNA bombardment, electroporation) and chemical (cationic lipids, cationic polymers) gene transport techniques are also described in this review.Reference 1 The drawbacks of using peptides in recombinant medicine, including limited bioavailability, instability, high production costs, clearance rates, and severe toxicity, are addressed via gene therapy.The intricacy and variety of tumors have presented challenges to global efforts in cancer prevention, early diagnosis, screening, and therapy (reviewed in [2]).Prognosis, chemotherapeutic effectiveness, and tumor growth are all determined by TME composition The application of gene therapy to address cancer molecular pathways has been spurred by growing understanding of the properties of tumor cells and the surrounding TME.Citation9Citation5 Gene therapy involves transferring genetic material (such as DNA or RNA) into the host organ by means of a vector.In vivo gene therapy involves introducing the genetic material into the target organ; ex vivo gene therapy involves altering host cells that are subsequently re-administered.While managing genetic diseases was initially the main goal of gene therapy, it is currently being used to treat a variety of conditions with various patterns of acquired and inherited disorders.A number of molecular approaches have emerged in recent decades that aid in editing DNA codes and modifying mRNA through post-transcriptional alterations.Reference Gene knockdown, deactivating problematic genes, inserting a new gene to treat a condition, and replacing dysfunctional genes with therapeutic genes are some of the ways that gene treatments work.Patient-derived tumor cells are extracted, often grown as 2D monolayers, genetically modified, and then reintroduced into the host in the ex vivo method [2].Approximately 2600 gene therapy trials are currently underway to treat a variety of ailments, and more than 3000 genes have been linked to mutations that cause disease.Lastly, this review provides a detailed summary of gene therapy medications that have been approved for the treatment of cancer, including names, indications, vectors, and gene therapy mode.Thus, gene products with safer vectors and improved biotechnologies are important for managing and preventing a variety of diseases in the future.Citation 8 A gene that expresses necessary therapeutic peptides, a plasmid-based gene encoding system that controls a gene's activity in the target organ, and a gene delivery system that controls the transfer of the encoding gene to host tissue make up gene delivery systems.Therapeutic nucleic acids (TNAs) administered ex vivo and/or in vivo have been shown to transfer genes into tumor cells (Figure 1) [20].These ex-vivo methods use immune cells that have been genetically modified to attack the tumor ??????????????????.cells after being extracted from the patient's blood