REVIEW ARTICLE

The war against cancer: Suicide gene therapy

Muzeyyen Izmirli1,2*, Dilara Sonmez2, Bulent Gogebakan1,2

1Mustafa Kemal University, School of Medicine, Department of Medical Biology, Hatay, Turkey
2Mustafa Kemal University, School of Medicine, Department of Molecular Biochemistry and Genetics, Hatay, Turkey

Abstract: The National Cancer Institute and the American Cancer Society announced that 1.6 million new cancer cases are projected to occur in the USA in 2016. One of the most innovative approaches against cancer is suicide gene therapy, in which suicide-inducing transgenes are introduced into cancer cells. When cancer treatments target the total elimination of tumor cells, there will be no side effects for normal cells. Cancer tissues are targeted through various targeted transport methods, followed by tissue-specific enzymes converting a systemically suitable prodrug into an active drug in the tumor. Suicidal genes are delivered by transporters, such as viral and non-viral vectors, into cancer cells. Suicide gene therapeutic strategies currently pursued are herpes simplex virus thymidine kinase gene with produg ganciclovir, cytosine deaminase gene, carboxyl esterase/irinotecan, varicella zoster virus thymidine kinase/6-methoxyuridine arabinonucleoside, nitroreductase Nfsb5-(aziridin-1-yl)-2,4-dinitrobenzamide, carboxypeptidase G2/4-(2-chloroethyl) (2-mesyloxyethyl)amino] benzoyl-L-glutamic acid, cytochrome p450-isosofosfamide, and cytochrome p450-cyclophosphamide. The goal of this review is to summarize the different suicide gene systems and gene delivery vectors addressed to cancer cells, with a particular emphasis on recently developed systems. Finally, we briefly describe the advantageous clinical applications and potential side effects of suicide gene therapy.

Keywords: suicide gene therapy; cancer; vector


*Correspondence to: Muzeyyen Izmirli, Mustafa Kemal University, School of Medicine, Department of Medical Biology, Hatay, Turkey; muzeyyenizmirli@gmail.com

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Introduction

In 2016, nearly 1.6 million new cases of cancer is projected to occur in the US[1]. Gene therapy delivers medicinal products via recombinant nucleic acid that regulates, repairs, replaces, adds, or deletes genetic sequences, which directly affects therapeutic, prophylactic, or diagnostic strategies. Gene therapy consists of two categories: germ line gene therapy and somatic gene therapy. Germ line gene therapy includes therapeutic or modified genes, which will be passed on to the next generation, while somatic gene therapy consists of genetic materials inserted into target cells but will not be passed on to the next generation[2]. Suicide gene therapy is a type of gene therapy using the insertion of genes into tumor cells from a viral or a bacterial gene, resulting in cancer cell death[3]. This therapy works on the basis of a cell’s self-destruction by a vector transferring the suicide gene that stimulates the apoptotic pathway with its product, thus allowing the conversion of a non-toxic prodrug into a lethal drug (Figure 1)[4]. This cancer therapy still remains as an integrated multimodality approach along with chemotherapy, radiotherapy, and surgery. Suicide gene therapy has been deemed safe and effective[5].

In this review, we discuss suicide gene therapy, summarizing the different suicide gene systems and gene delivery vectors. Moreover, we highlight several strategies that have been used in combination with suicide gene therapy and believe these approaches would be a useful resource in the future for new ideas.
The war against cancer: Suicide gene therapy

**Figure 1.** Mechanism of the HSV-TK suicide gene therapy system. (1) Suicide genes transferred into viral vectors and infected tumor cells; (2) Expression of the suicide gene and formation of the enzyme product; (3) The combination of the prodrug and enzyme caused the conversion from non-toxic to toxic drug; (4) Owing to the apoptotic pathway, tumor cells are destroyed, induced by a lethal drug.

The mechanisms of suicide gene therapy

Suicide gene therapy consists of different suicide systems. The most studied systems are herpes simplex virus thymidine kinase gene (HSV-TK) with prodrug ganciclovir (GCV), cytosine deaminase gene (CD), carboxyl esterase/irinotecan (CE/CPT-11), varicella zoster virus thymidine kinase/6-methoxypurine arabinonucleoside (Vztk/araM), nitroreductase N6sh/5-(aziridin-1-yl)-2, 4-dinitrobenzamide (NTR/CB1954), carboxypeptidase G2/4-[(2-chloroethyl)(2-mesyloxyethyl) amino] benzoyl-L-glutamic acid (CPG2/CMDA), cytochrome p450-ifsomamide, and cytochrome p450- cyclophosphamide. Additionally, there are other systems that are not widely used such as the purine nucleoside phosphorylase (PNP), linamarase, β-galactosidase, and nitroreductase.

**Herpes simplex virus thymidine kinase/ganciclovir (HSV-TK/GCV).** HSV-TK catalyzes the phosphorylation of GCV into GCV monophosphate. Cellular kinases then convert GCV monophosphate to GCV triphosphate, which is an analogue of deoxyguanosine triphosphate. GCV triphosphate incorporates DNA polymerase and this causes the termination of DNA replication and subsequently, apoptosis. The potential immunogenicity of the viral enzyme limits the HSV/TK-GCV suicide system. However, GCV triphosphate passively enters cells via gap junctions, which can potentially limit the overall therapeutic effects. Incorrect GCV triphosphate incorporation into DNA leads to a delayed S-phase and the activation of several exonucleases and repair mechanisms of the G2-phase arrest cell cycle (Figure 2).

Previous studies have found that apoptosis was induced by HSV-TK/GCV in several tumor cells including melanoma, glioma, leukemia, bladder cancer, intrahepatic metastasis of liver cancer, and prostate cancer.

**Figure 2.** The HSV-TK suicide gene therapy system.
**Varicella zoster virus thymidine kinase/6-methoxy-purine arabinonucleoside (Vztk/ araM).** The varicella zoster virus also expresses thymidine kinase (VZV-TK) that is responsible for the activation of anti-herpetic nucleoside acyclovir. 6-Methoxy purine arabinonucleoside (araM) plays the role of a prodrug for the VZV-TK enzyme. araM is monophosphorylated by VZV-TK, resulting in araM monophosphate (araMMP). Four cellular enzymes – AMP deaminase, adenyl succinate synthetase, AMP kinase, and nucleoside diphosphate kinase – metabolize into the highly toxic adenine arabinonucleoside triphosphate (araATP). The VZV-TK suicide gene system has generally poor efficacy on the activated prodrug against some cancer cell types. However, it has been recently developed as improved potency anti-herpetic pyrimidine nucleoside analogues, including the novel prodrug (E)-5-(2-bromo vinyl)-2-deoxyuridine (BVDU) which is 80-fold more toxic to cells[6].

**Cytosine deaminase/5-FC system (CD/5-FC).** Cytosine deaminase is expressed by bacteria and yeast but not in mammalian cells. The CD enzyme catalyzes the hydrolytic deamination of cytosine into uracil. Hence, it converts 5-FC (non-toxic) to 5-FU (toxic), an important drug used in conventional cancer chemotherapy. It causes apoptosis via three pathways: thymidylate synthase inhibition, formation of 5-FU RNA, and formation of 5-FU DNA complexes[12]. 5-FU enters the nucleotide pathway and is converted to 5-fluoro-2'-deoxyuridine-5'-monophosphate (5-FdUMP), 5-fluorouridine-diphosphate (5-FUDP), and 5-fluorouridine-triphosphate (5-FUTP). 5-FdUMP is an irreversible inhibitor of thymidylate synthase, resulting in the limitation of thymidine and inhibition of DNA synthesis. 5-FUDP is also converted to 5-FdUTP, which can be inserted into DNA to cause DNA damage and cell death. 5-FUTP can also be incorporated into RNA, replacing UTP and inhibiting RNA processing[3]. Previous studies have shown that apoptosis was induced by the CD/5-FC suicide system in several tumor cells, including in colon cancer (Figure 3)[17]. A uracil phosphoribosyltransferase (UPRT) gene, when inserted into the CD/5-FC system, improves its[18]. Importantly, this CD-UPRT/5-FC suicide system has been shown to be effective against 5-FU-resistant cancer cells. CD activity is highly increased by the FCU protein[19]. A number of clinical research have shown antitumor activities of the CD/5-FC suicide system in fibrosarcoma[20], glioma[21], breast cancer[22], prostate cancer[23], and metastatic formations of different origin[24].

**Carboxyl esterase/irinotecan system (CE/CPT-11).** Carboxyl esterase is a serine esterase, expressed in several tissues including serum, liver, and the intestines[25]. Carboxyl esterase participates in the metabolism of toxins or drugs and the resulting carboxylates are conjugated by other enzymes. This enhances the general bio-availability of many therapeutic agents. Irinotecan (or CPT-11) is a chemotherapy agent that is cleaved by carboxyl esterase to generate a potent antitumor effect via 7-ethyl-10-hydroxycamptothecin (SN-38), an inhibitor of topoisomerase I activity[26]. SN-38 is not soluble and only acts locally. It inhibits topoisomerase I, resulting in the accumulation of double-stranded DNA breaks in actively dividing cancer cells. This would then inhibit DNA replication and transcription. A preclinical study has found that CE/CPT-11 suicide system reduces tumor size[27].

**NitroreductaseNfsB/5-(aziridin-1-yl)-2,4-dinitrobenzamide system (NTR/CB1954).** Nitroreductase NfsB (NTR) is a flavoprotein, which plays a role by reducing DNA damage and cell death. 5-FUTP can also be incorporated into RNA, replacing UTP and inhibiting RNA processing[3]. Previous studies have shown that apoptosis was induced by the CD/5-FC suicide system in several tumor cells, including in colon cancer (Figure 3)[17]. A uracil phosphoribosyltransferase (UPRT) gene, when inserted into the CD/5-FC system, improves its[18]. Importantly, this CD-UPRT/5-FC suicide system has been shown to be effective against 5-FU-resistant cancer cells. CD activity is highly increased by the FCU protein[19]. A number of clinical research have shown antitumor activities of the CD/5-FC suicide system in fibrosarcoma[20], glioma[21], breast cancer[22], prostate cancer[23], and metastatic formations of different origin[24].

**Figure 3.** The CD/5-FC suicide gene therapy system
quinones and nitro aromatics via NADPH or NADH. The prodrug 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB1954) is catalyzed by NTR, resulting in the production of a potent cellular cytotoxic agent [28]. Previous studies have found the presence of cytotoxic agents of the NTR/CB1954 system in several tumor cells including in prostate cancer [39].

**Carboxypeptidase G2/4-[(2-chloroethyl)(2-mesyloxyethyl) amino] benzoyl-L-glutamic acid system (CPG2/CMDA).** Carboxypeptidase G2 (CPG2) is a bacterial enzyme which cleaves glutamic acid from the prodrug 4-[(2-chloroethyl)(2-mesyloxyethyl) amino] benzoyl-L-glutamic acid (CMDA). CPG2 gene mutants are created from the result of targeting CPG2 to the cell membrane and the expression of cell surface protein. CPG2 causes the production of a DNA cross-linking mustard drug, 4-[(2-chloroethyl)(2-mesyloxyethyl) amino] benzoic acid, which acts as a potent cytotoxic agent. Several cells expressing CPG2 are sensitive to CMDA, but some tumors are resistant to this toxin owing to limited uptake of the prodrug [39].

**Vectors and cargo elements**

Once a suicide gene system has been elected, the suicide gene must be selectively transferred to the tumor cells [30]. Vectors enter via receptor-mediated endocytosis or membrane fusions. Targeted vectors require specific domains, which facilitate entries into the cytoplasm and nucleus via pathways that include endosomal, lysosomal, or nuclear pore complex. In designing suicide gene therapies, these determinative parameters affect the choice of vectors [6]. The optimal transporter should have minimum side effects and higher specificity, and would efficiently transfer genes to the target cells [30].

In summary, a vector has to have the ability to deliver. First of all, it must protect DNA from enzymatic degradation and must have receptors for specific targets on the cell membrane. When a vector passes into target cells, it must degrade its own membrane and deliver the cargo smoothly [31].

There are several transfer methods by using vectors, such as viral and non-viral vectors [30], [30].

**Viral vectors**

Viruses are perfect vectors to transfer foreign DNA. It replicates its genome in host cell by integrating with host DNA, or via plasmid replication if it has a region of origin. Viral-based vectors are efficient means to transfer suicide genes into cancer cells. These vectors are designed by introducing therapeutic genes involved in viral replication or pathogenic protein production with foreign therapeutic genes. The selection of viral system depends on several parameters: characteristics of the tumor type, suicide gene system, and therapeutic strategy. There are numerous viral vectors such as adenoviruses, retroviruses, vaccinia viruses, poxviruses, adeno-associated viruses, herpes simplex viruses, and lentiviruses [32].

**Adenoviruses.** The most popular viral vector owing to their ability to introduce dividing and non-dividing cells [33]. Adenoviruses are DNA viruses without envelopes, consisting of double stranded linear DNAs and capsids. When adenoviruses enter the target cell via adenovirus receptor-mediated endocytosis into nasal, tracheal, and pulmonary epithelia, it is transported to the nucleus where viral genes and proteins are expressed. The suicide gene is inserted into the adenovirus genome, leading to the expression of the inserted gene in host cells (cancerous cells). However, the main challenge of this approach is that gene expression is temporary because adenoviruses do not integrate into the target cell genome. New methods, such as arming chimeric adenovirus-based vectors with the capacity to integrate into target cells’ genome, should be developed. There is also a serious problem with adenoviral-based gene therapy as it has the ability to arouse lethal and intense immune reactions in some patients, causing ornithine transcarbamylase deficiency and resulting in the death of a study patient due to an anaphylactic shock [34].

**Herpes simplex virus (HSV).** HSV has an envelope encasing its DNA. It enters target cells by receptor-mediated ligand glycoproteins (including gB, gH, and gL) into oral, ocular, genital epithelia, and nervous system cells [6].

**Lentivirus.** Lentivirus is a retrovirus with a single stranded RNA encased in an envelope. It has the ability to introduce dividing and non-dividing cells. The human immunodeficiency virus (HIV) is the most recognized member of lentiviruses. The ligands of the lentivirus bind to cluster differentiation 4 (CD4) receptors, which exist on CD4+ T cells, macrophages, and dendritic cells. The viral RNA is reverse transcribed into double-stranded DNA, which enters into the nucleus and is inserted into the target DNA [6].

**Non-viral vectors**

There are several non-viral vectors, including physical vectors, cellular vectors, and inorganic vectors [35].

**Physical vectors**

Physical vectors include the injection of naked
DNA, ballistic DNA injection, electroporation, sonoporation, magnetofection, and hydroproporation.

**Injection of Naked DNA.** Naked DNA or plasmid injection is a type of non-viral vector. Direct injection of free DNA into targeted tissues (tumor tissues) has been shown to produce high levels of gene expression and causes the expression of tumor antigens that can function as a cancer vaccine. This method is simple and exhibits low cellular uptake. In short, the injection of naked DNA is used when low gene transfer efficiency can be changed by the activation of immunity. On the other hand, naked DNA is not suitable for systemic administration because of serum nucleases. Moreover, direct injection of naked DNA has other limitations – it is suitable for only a few applications involving tissues that are easily accessible to direct injection such as skin and muscle cells.

**Ballistic DNA injection.** This technology is also known as the gene gun, particle bombardment or microprojectile, and is a gene transfer technique that was first used in plants. Ballistic DNA injection consists of passing through the target tissue with DNA-coated heavy metal particles at adequate speeds. The metal particles could comprise silver, gold, or tungsten. Its advantage is the sensitive delivery of DNA doses. On the other hand, the drawback of this technique is the short-term expression of transferred genes and cellular damage.

**Electroporation.** This technique is based on loading the cell membrane with high capacity electrical field for a nanosecond to open a pore. During this time, DNA molecules could pass through the pore in approximately ten seconds. However, cells can be damaged due to pulse duration and the severity of the electrical field during electroporation. Electrical strength must be about 700 V/cm while the pulses must be in micro- or milliseconds.

**Sonoporation.** Sonoporation changes the permeability of cell plasma membrane with sonication. Therapeutic gene is delivered via a microbubble, of which its outer shell is covered with lipids, proteins, or synthetic biopolymers. The interior is suffused with air/nitrogen/inert gas of high-molecular weight. Microbubbles are transferred with the therapeutic gene and circulated into the blood. Sonic waves bump with intensity in pulses over the target tissue.

**Magnetofection.** This technique is based on an in vitro research using magnetic nanoparticle-transferred therapeutic genes. This system captures and holds target cells with a magnet. Therapeutic genes can pass through cell membranes when the pulses of magnetic nanoparticles cause the degradation of cell membrane, enzymatic cleavage of cross-linking molecule, or charge interaction.

**Hydroporation.** This technique injects DNA solutions into the target tissue, which penetrates the cell membrane via hydrodynamic pressure. In literature, this technique has been used for gene therapy research of hepatic cells.

**Cellular vectors**

Cellular vectors consist of bacterial and mammalian cell vectors.

**Bacterial vectors.** Commonly used bacteria are obligate or facultative anaerobic bacteria such as strains of Clostridia, Bifidobacteria, and Salmonella. These have the ability to infect hypoxic areas of tumors and have presented efficient antitumor activity against chemoresistant cancer cell lines. Bacterial vectors can be modified to deliver bacterially-expressed therapeutic genes.

**Mammalian cell vectors.** Several mammalian cells exhibiting tumor tropism have been recently suggested as transporters for gene therapy, such as bone marrow stromal cells (MSC), neural stem cells (NSC), endothelial progenitor precursor, and blood outgrowth endothelial cells. MSCs are pluripotent progenitor cells, which maintain and regenerate diverse tissues after injury and during chronic inflammation. These features have led to the use of MSCs as gene delivery transporters for tumor cells. NSCs have an inherent tumor tropism and are therefore reliable delivery agents for target therapeutic gene products to primary and metastatic tumors. Blood outgrowth endothelial cells are withdrawn to the tumor vasculature. In addition, erythrocyte ghosts are also used as vectors for suicide gene therapy.

**Inorganic vectors**

**Calcium phosphate, silica, gold, and several magnetic compounds.** These are inorganic particles that are used as vectors for gene transport. Their structures have different formats and porousities. An example of biocompatible structures is silica-coated nanoparticles, which can be used for gene therapy. There are several studies that highlight silica and magnetic inorganic nanoparticles (such as Fe₃O₄ and MnO₂) for gene delivery. One of the main candidates that is currently investigated for gene therapy is gold nanoparticles, which are recommended for transferring nucleic acids into tumors.

**Nanoparticles.** A novel form of gene transfer vehicle is nanoparticles that include a cationic core. They have been investigated as effective suicide gene vectors for gene therapy. Nanoparticles have the ability to diffuse within the tumor tissue via passive transport and have enhanced effectiveness.
permeability and retention effect in tumor tissues. This is a combined result of an increased permeability of tumor blood vessels and a decreased rate of clearance within the tumor. This method is usually used for interference RNA delivery but not for DNA plasmid delivery.

**Synthetic or natural biodegradable particles.** Synthetic or natural biodegradable particles are composed of three subtypes, which are: polymeric-based vectors [poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLA), chitosan, dendrimers, polyethacrylates], cationic lipid-based vectors (cationic liposomes, cationic emulsions, solid lipid nanoparticles), and peptide-based vectors (poly-L-lysine, protamine). Cationic liposomes and cationic liposomes are commonly used as non-viral gene delivery methods to deliver genes into cells. Negatively charged liposomes can interact spontaneously with negatively charged DNA to form DNA clusters of aggregated vesicles along the nuclear acid. Liposomes with suicidal genes can interact with negatively charged cell membranes. The interaction of DNA and liposomes, or polyplexes, leads to the formation of lipoplexes.

Liposomes have some advantages including the capacity to transport large amounts of genetic material. Their physico-chemical versatility allows innumerous modifications and liposomes can be easily and inexpensively produced on a large scale. Moreover, liposomes are safer than viral vectors owing to their reduced chance for insertional mutagenesis or side effects from immune reactions to the vector. However, liposomes have some disadvantages including poor levels of transfection, considerable reduction of biological activity by serum components, and toxic effects. Gene transport using liposomes was first demonstrated using a synthetic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl ammonium chloride (DOTMA). DOTMA contains unilamellar liposomes, which interact with DNA to form lipid-DNA complexes. Subsequently, DOTMA facilitates the fusion of suicide gene complex with the plasma membrane of target cells, resulting in both uptake and expression of the DNA.

**Cancer-specific promoters**

There are several cancer-specific promoters, including epidermal growth factor receptor (EGFR), human epidermal growth factor receptor/neu (Her2/Neu), carcinoembryonic antigen (CEA), vascular endothelial growth factor receptor (VEGFR), cluster differentiation 71 (CD71), CD44, CD133, breast cancer 1 (BRCA1), BRCA2, stage-specific embryonic antigen-4 (SSEA-4), prostate-specific antigen (PSA), folate receptor (FR), transferrin receptor (TfR), mucins, tumor resistance anti-

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**EGFR (ErbB1).** This receptor is present on healthy and cancerous cells. The number of EGFR receptors may differ on cells. The number of EGFR receptors is approximately $3 \times 10^7$ on healthy glial cells and approximately $2-3 \times 10^6$ on tumor cells. The recombinant adenoviral vector and genetically engineered variable fragment antibodies target EGFR receptors of ovarian and breast cancers.

**Her2 (ErbB2).** This is a cancer biomarker that is shed by tumor cells into the blood, and thus is routinely detected. It is also the target for vectors delivering therapeutic genes.

**Folic acid receptor (FR).** Folic acid plays a role in the synthesis and repair of genomic DNA and RNA, as well as methylation reactions. Folic acid cannot directly penetrate the cell membrane. In fact, it penetrates by endocytosis through the FR. Hence, FR is absolutely over-expressed on the surfaces of various tumor types including ovarian, kidney, lung, brain, endometrial, colorectal, pancreatic, gastric, prostate, testicular, bladder, head and neck, breast, and lung cancers. Therefore, FR is viewed as a therapeutic target that may provide an effective option for targeted suicide cancer therapy.

**Cluster of differentiation 44 (CD44).** This is present on cells from a variety of tissues, including those of epithelial origin such as prostate, ductal epithelium of the breast, mucosa, lungs, and bladder of cancerous cells. CD44 variant 6 and CD133 are specific to epithelial cancers and are used as lentivirus-driven suicidal genes.

**Transferrin receptor (TfR).** Transferrin carries iron into cells, which in turn express the TfR. Iron-bound Tf is transported by TfR, which is over-expressed in tumor cells. One of the therapeutic strategies of suicide cancer therapy is to decrease the level of iron.

**Mucins.** Mucins are glycosylated proteins on cell surfaces and their major function involves imparting protection against inflammation, bacteria, virus, pollutants, and pH, among other things. The structure and localization of
mucins are specific for every cell type, and mucins play key roles in cell-cell and cell-extracellular matrix interactions as well as in cell signaling for cancerous cells. Specific expression of mucins is important for tumor progression and therefore can be used as biomarkers and therapeutic targets in epithelial cancers. Moreover, mucins are present on breast, pancreatic, and ovarian cancer cells. MUC-1/Z is also a form of mucin and is exploited for the production of specific antibodies. These antibodies lead as vectors with the therapeutic transporter to cancer cells.

**Telomerase.** Telomerase is a form of RNA polymerase and is generally over-expressed in cancerous cells. Therefore, it is used as a promoter of suicidal genes in cancer cells. For example, such strategies involve the transduction of ovarian cancers with HSV-TK under the telomerase promoter.

**Cytokeratin 18 and 19 (CK18-19).** Cytokeratins are present in epithelial cells and are biomarkers of the neoplasms of epithelial cells. They can occur during tumor genesis and metastasis. Moreover, cytokeratins are biomarkers of differentiation into one of the three germ layers for human embryonic stem cells.

**Stage-specific embryonic antigen-4 (SSEA-4) and tumor resistance antigen 1-60 (TRA-1-60).** SSEA-4 is a glycoprotein and is expressed in embryonic stem cells during embryonic development. It is identified as a biomarker for cancerous embryonic testes and ovaries. TRA-1-60 is also a marker for human embryonic stem cells and carcinomatous cells. TRA-1-60 is also suggested as a biomarker for testes and ovarian cancer stem cells. These biomarkers of pluripotency are expressed upon the differentiation of cells.

**Cyclooxygenase (Cox).** It is also known as prostaglandin-endoperoxide synthase (PTGS) and responsible for catalyzing prostaglandins, prostacyclin, and thromboxane. Aspirin inhibits Cox. Due to the expression of the Cox gene in colorectal cancer, it is used as a biomarker in suicide gene therapy.

**Chimeric antigen receptors (CAR).** CARs are cell surface antigens and are known as artificial T-cell receptors and chimeric immune receptors. CARs present another autologous cell-based therapy aiming at tumor-associated cell surface antigens. CARs are engineered receptors and add an optional specificity onto an immune effector cell. Therefore, CARs are composed of parts from different sources. This approach brings together the permanence of cytotoxic T-cells with the specificity of monoclonal antibodies and expansion potential.

**Vascular endothelial growth factor (VEGF).** VEGF is a growth factor and it stimulates vasculogenesis and angiogenesis. VEGF is expressed by cancer cells and it stimulates the proliferation of endothelial and cancerous cells. The inhibition of angiogenesis and vasculogenesis is a new target for cancer in suicide gene therapy. Liu et al. investigated a model of suicide gene therapy system using anti-VEGF with a triple-gene vector expressing VEGF-shRNA and CD/TK.

**Clinical applications**

Gene therapy is a good alternative to conventionally used medical therapies and holds a great promise for the treatment of various diseases, especially cancer. At present, most of the clinical applications in suicide gene therapy have been aimed at the treatment of cancer (64.4% of all gene therapies). Suicide gene therapies have targeted several cancers including lung, gynecological, skin, urological, neurological, and gastrointestinal tumors as well as hematological malignancies and pediatric tumors.

**Glioblastoma multiforme (GBM) and medulloblastoma.** GBMs and medulloblastomas are the most regularly found and most aggressive form of brain tumors in adults. GBM treatment involves conventional cancer therapy including chemotherapy, radiotherapy, and surgery as well as suicide gene therapy. Eight patients with GBM were treated with a HSV-TK gene-bearing liposomal vector, followed by the systemic application of GCV. Connexin 43 (Cx43), which is differently expressed in different glioblastoma cell lines, was used as a biomarker. This therapy resulted in a greater than 50% reduction of tumor volume in two patients and showed local effects in the remaining patients.

**Mesothelioma and lung cancer.** Mesothelioma is a deadly cancer of the pleura. In a previous study, human PA1STK cell line transduced with the HSV-TK gene was directly infused into malign pleural effusions and subsequently, GCV was infused intravenously for seven days. However, this study did not achieve the desired suicide therapy effect.

Lung cancer is the deadliest type of cancer and this has facilitated the growth of targeted cancer suicide gene therapy trials. CD/5-FC suicide and NTR/CB1954 systems are used on small cell lung cancer (SCLC). In this cancer, insulinoma-associated 1 (INSM1) promoter and nuclear factor kappa B (NFκB) are used as biomarkers.

**Gastrointestinal cancers.** These cancers include gastric, hepatocellular, pancreatic, and colon cancers. Gastric cancer is the fourth most common type of cancer and the second highest cause of death. Hepatocellular carcinoma is the sixth most prevalent cancer and the third most frequent cause of cancer-related death. Many Americans are affected by pancreatic cancer and a
A growing number of people are diagnosed with colon cancer[82]. Suicide gene systems including HSV-TK have been used to treat gastrointestinal cancers. Several biomarkers including CEA, CD44, CD133, and hTERT are investigated on gastrointestinal cancer cells[80]. These biomarkers facilitate early diagnosis for the abovementioned cancer types and are targets for diagnostic molecular imaging and targeted therapies[41,81].

**Gynecological and andrological cancers.** These include prostate cancer, endometrial cancer, and ovarian cancer. Prostate cancer is the second leading cause of death in men in the United States[1]. There are numerous studies on HSV-TK suicide genes, 5-FC+GCV prodrug therapy, NTR/CB1954, and nitro reductase system on prostate cancer. Several biomarkers including prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), androgen, C-X-C chemokine receptor type 4 (CXCR4), and epithelial cell adhesion molecule (EpCAM) have been identified for prostate cancer[82–84]. Consequently, promoters of the aforementioned genes are incorporated into the constructs of cancer suicide genes, which are expressed in prostate cancer cells[61,65].

Endometrial cancer is the most common cancer affecting the gynecologic system and ovarian cancer is the deadliest cancer among all female gynecologic cancers[85]. HSV-TK suicide gene system and SSEA-4, TRAIL-1-60, CD44, CD133, BRCA1 as well as BRCA2 biomarkers are used in endometrial and ovarian cancer therapies[55].

**Breast cancer.** Breast cancers are diagnosed in about 229,060 women in the USA[86]. HSV-TK suicide gene system and Her2/neu proto-oncogene, along with the epidermal growth factor variant III (EGFRvIII) biomarkers, are used for breast cancer suicide gene therapy[54].

**Conclusion**

Currently, there are different available vectors and several delivery and effector systems for suicide gene therapy, and these techniques are being used for cancer therapy. Suicide gene therapy has demonstrated that a drug that is injected at a distant site of the primary tumor tissue can still efficiently affect the primary tumor. Moreover, suicide gene therapy enhances the immune system’s response on primary and metastatic tumor tissues. However, there are obvious problems associated with the therapy, which include the activation of specific prodrugs, introduction of suicide genes into cancerous cells without damaging healthy cells, and host immune response to the vector and suicide gene product[54]. Furthermore, preclinical applications have only been undertaken in animal models for short periods. Thus, the lifetime risk of mutagenesis in a human is difficult to predict[87]. In conclusion, suicide gene therapy is a hopeful approach in overcoming cancer. Highly specific research efforts are necessary to address these existing shortcomings in developing suicide gene therapy into a real benefit for cancer patients.

**Conflict of interest**

The authors declare no potential conflict of interest with respect to the research, authorship and/or publication of this article.

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